PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/47, 16/24

A1

(11) International Publication Number: WO 96/12735

(43) International Publication Date: 2 May 1996 (02.05.96)

(21) International Application Number: PCT/US95/12724

(22) International Filing Date: 12 October 1995 (12.10.95)

(30) Priority Data:

08/327,514	19 October 1994 (19.10.94)	US
08/494,440	19 June 1995 (19.06.95)	US
08/533,901	26 September 1995 (26.09.95)	US

(71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US).

(72) Inventors: LIN, Lih-Ling; 107 College Road, Concord, MA 01742 (US). CHEN, Jennifer; 833 West Roxbury Parkway, Chestnut Hill, MA 02167 (US). SCHIEVELLA, Andrea, R.; 39 Edward Street, Winchester, MA 01890 (US). GRAHAM, James; 28 Foskett Street, Somerville, MA 02144 (US).

(74) Agent: BROWN, Scott, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).

(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL TNF RECEPTOR DEATH DOMAIN LIGAND PROTEINS AND INHIBITORS OF LIGAND BINDING

(57) Abstract

Novel TNF receptor death domain ("TNF-R1-DD") ligand proteins are disclosed. Polynucleotides encoding the TNF-R1-DD ligand protein are also disclosed, along with vectors, host cells, and methods of making the TNF-R1-DD ligand protein. Pharmaceutical compositions containing the TNF-R1-DD ligand protein, methods of treating inflammatory conditions, and methods of inhibiting TNF-R death domain binding are also disclosed. Methods of identifying inhibitors of TNF-R death domain binding and inhibitors identified by such methods are also disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

NOVEL TNF RECEPTOR DEATH DOMAIN LIGAND PROTEINS AND INHIBITORS OF LIGAND BINDING

This application is a continuation-in-part of application Ser. No. 08/494,440, filed June 19, 1995, which was a continuation-in-part of application Ser. No. 08/327,514, filed October 19, 1994.

BACKGROUND OF THE INVENTION

The present invention relates to the field of anti-inflammatory substances and other substances which act by inhibiting binding to the intracellular domain of a tumor necrosis factor receptor (hereinafter "TNF-R"), such as, for example, the P55 type (or TNF-R1) TNF receptor. More particularly, the present invention is directed to novel ligands which bind to the TNF-R intracellular domain and to inhibition or modulation of signal transduction by this receptor.

Tumor necrosis factor (herein "TNF") is a cytokine which produces a wide range of cellular activities. TNF causes an inflammatory response, which can be beneficial, such as in mounting an immune response to a pathogen, or when overexpressed can lead to other detrimental effects of inflammation.

The cellular effects of TNF are initiated by the binding of TNF to its receptors (TNF-Rs) on the surface of target cells. The isolation of polynucleotides encoding TNF-Rs and variant forms of such receptors has been described in European patent publication Nos. EP 308,378, EP 393,438, EP 433,900, EP 526,905 and EP 568,925; in PCT patent publication Nos. WO91/03553 and WO93/19777; and by Schall *et al.*, Cell *61*:361-370 (1990) (disclosing the P55 type TNF receptor). Processes for purification of TNF-Rs have also been disclosed in U.S. Patent No. 5,296,592.

Native TNF-Rs are characterized by distinct extracellular, transmembrane and intracellular domains. The primary purpose of the extracellular domain is to present a binding site for TNF on the outside of the cell. When TNF is bound to the binding site, a "signal" is transmitted to the inside of the cell through

the transmembrane and intracellular domains, indicating that binding has occurred. Transmission or "transduction" of the signal to the inside of the cell occurs by a change in conformation of the transmembrane and/or intracellular domains of the receptor. This signal is "received" by the binding of proteins and other molecules to the intracellular domain of the receptor, resulting in the effects seen upon TNF stimulation. Two distinct TNF receptors of ~55 kd ("TNF-R1") and ~75 kd ("TNF-R2") have been identified. Numerous studies with anti-TNF receptor antibodies have demonstrated that TNF-R1 is the receptor which signals the majority of the pleiotropic activities of TNF. Recently, the domain required for signaling cytotoxicity and other TNF-mediated responses has been mapped to the ~80 amino acid near the C-terminus of TNF-R1. This domain is therefore termed the "death domain" (hereinafter referred to as "TNF-R death domain" and "TNF-R1-DD") (see, Tartaglia *et al.*, Cell *74*:845-853 (1993)).

While TNF binding by TNF-Rs results in beneficial cellular effects, it is often desirable to prevent or deter TNF binding from causing other detrimental cellular effects. Although substantial effort has been expended investigating inhibition of TNF binding to the extracellular domain of TNF-Rs, examination of binding of proteins and other molecules to the intracellular domain of TNF-Rs has received much less attention.

However, ligands which bind to the TNF-R intracellular domain have yet to be identified. It would be desirable to identify and isolate such ligands to examine their effects upon TNF-R signal transduction and their use as therapeutic agents for treatment of TNF-induced conditions. Furthermore, identification of such ligands would provide a means for screening for inhibitors of TNF-R/intracellular ligand binding, which will also be useful as anti-inflammatory agents.

SUMMARY OF THE INVENTION

Applicants have for the first time identified novel TNF-R1-DD ligand proteins and have isolated polynucleotides encoding such ligands. Applicants have also identified a known protein which may also bind to the death domain of TNF-R.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide encoding a protein having TNF-R1-DD ligand

protein activity. In preferred embodiments, the polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2;

(e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;

(f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3;

(g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;

(h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4;

(i) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;

(j) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9;

(k) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;

(l) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10;

(m) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;

(n) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11:

(o) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12:

(p) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12;

(q) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 3 to nucleotide 2846;

(r) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:13, which encodes a protein having TNF-R1-DD ligand protein activity;

(s) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:14;

(t) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 and having TNF-R1-DD ligand protein activity; and

(u) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(t).

In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with such polynucleotide compositions.

Processes are also provided for producing an TNF-R1-DD ligand protein, which comprises:

(a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium: and

(b) purifying the TNF-R1-DD ligand protein from the culture.

The ligand protein produced according to such methods is also provided by the present invention.

Compositions comprising a protein having TNF-R1-DD ligand protein activity are also disclosed. In preferred embodiments the protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) fragments of the amino acid sequence of SEQ ID NO:2;

(c) the amino acid sequence of SEQ ID NO:4;

(d) fragments of the amino acid sequence of SEQ ID NO:4;

(e) the amino acid sequence of SEQ ID NO:6;

(f) fragments of the amino acid sequence of SEQ ID NO:6;

(g) the amino acid sequence of SEQ ID NO:10;

(h) fragments of the amino acid sequence of SEQ ID NO:10;

(i) the amino acid sequence of SEQ ID NO:12;

(j) fragments of the amino acid sequence of SEQ ID NO:12;

(k) the amino acid sequence of SEQ ID NO:14; and

(l) fragments of the amino acid sequence of SEQ ID NO:14;

the protein being substantially free from other mammalian proteins. Such compositions may further comprise a pharmaceutically acceptable carrier.

Compositions comprising an antibody which specifically reacts with such TNF-R1-DD ligand protein are also provided by the present invention.

Methods are also provided for identifying an inhibitor of TNF-R death domain binding which comprise:

(a) combining an TNF-R death domain protein with an TNF-R1-DD ligand protein, said combination forming a first binding mixture;

(b) measuring the amount of binding between the TNF-R death domain protein and the TNF-R1-DD ligand protein in the first binding mixture;

(c) combining a compound with the TNF-R death domain protein and an TNF-R1-DD ligand protein to form a second binding mixture;

(d) measuring the amount of binding in the second binding mixture; and

(e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture;

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the amount of binding of the second binding mixture occurs. In certain preferred embodiments the TNF-R1-DD ligand protein used in such method comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) fragments of the amino acid sequence of SEQ ID NO:2;

(c) the amino acid sequence of SEQ ID NO:4;

(d) fragments of the amino acid sequence of SEQ ID NO:4;

(e) the amino acid sequence of SEQ ID NO:6;

(f) fragments of the amino acid sequence of SEQ ID NO:6;

(g) the amino acid sequence of SEQ ID NO:8;

(h) fragments of the amino acid sequence of SEQ ID NO:8

(i) the amino acid sequence of SEQ ID NO:10;

(j) fragments of the amino acid sequence of SEQ ID NO:10;

(k) the amino acid sequence of SEQ ID NO:12;

(l) fragments of the amino acid sequence of SEQ ID NO:12;

(m) the amino acid sequence of SEQ ID NO:14; and

(n) fragments of the amino acid sequence of SEQ ID NO:14.

Compositions comprising inhibitors identified according to such method are also provided. Such compositions may include pharmaceutically acceptable carriers.

Methods are also provided for preventing or ameliorating an inflammatory condition which comprises administering a therapeutically effective amount of a composition comprising a protein having TNF-R1-DD ligand protein activity and a pharmaceutically acceptable carrier.

Other embodiments provide methods of inhibiting TNF-R death domain binding comprising administering a therapeutically effective amount of a composition comprising a protein having TNF-R1-DD ligand protein activity and a pharmaceutically acceptable carrier.

Methods are also provided for preventing or ameliorating an inflammatory condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a protein selected from the group consisting of insulin-like growth factor binding protein-5 ("IGFBP-5"), and fragments thereof having TNF-R1-DD ligand protein activity. Such proteins may also be administered for inhibiting TNF-R death domain binding.

Methods of preventing or ameliorating an inflammatory condition or of inhibiting TNF-R death domain binding are provided. which comprise administering to a mammalian subject a therapeutically effective amount of inhibitors of TNF-R death domain binding. are also provided.

Methods of identifying an inhibitor of TNF-R death domain binding are also provided by the present invention which comprise:

(a) transforming a cell with a first polynucleotide encoding an TNF-R death domain protein, a second polynucleotide encoding an TNF-R1-DD ligand protein, and at least one reporter gene, wherein the expression of the reporter gene is regulated by the binding of the TNF-R1-DD ligand protein encoded by the second polynucleotide to the TNF-R death domain protein encoded by the first polynucleotide;

(b) growing the cell in the presence of and in the absence of a compound; and

(c) comparing the degree of expression of the reporter gene in the presence of and in the absence of the compound;

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the degree of expression of the reporter gene occurs. In preferred embodiments, the cell is a yeast cell and the second polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1, which encodes a protein having TNF-R1-DD ligand protein activity;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 and having TNF-R1-DD ligand protein activity;

(e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;

(f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3. which encodes a protein having TNF-R1-DD ligand protein activity;

(g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;

(h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 and having TNF-R1-DD ligand protein activity;

(i) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 2 to nucleotide 559;

(j) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5, which encodes a protein having TNF-R1-DD ligand protein activity;

(k) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:6;

(l) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 and having TNF-R1-DD ligand protein activity;

(m) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 57 to nucleotide 875;

(n) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7, which encodes a protein having TNF-R1-DD ligand protein activity;

(o) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:8;

(p) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 and having TNF-R1-DD ligand protein activity;

(q) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;

(r) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9;

(s) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;

(t) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10;

(u) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;

(v) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11;

(w) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12;

(x) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12;

(y) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 3 to nucleotide 2846;

(z) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:13, which encodes a protein having TNF-R1-DD ligand protein activity;

(aa) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:14;

(bb) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 and having TNF-R1-DD ligand protein activity; and

(cc) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(bb), which encodes a protein having TNF-R1-DD ligand protein activity.

BRIEF DESCRIPTION OF THE FIGURES

Figs. 1 and 2 depict autoradiographs demonstrating the expression of TNF-R1-DD ligand proteins of the present invention.

Fig. 3 depicts an autoradiograph demonstrating the expression of clones 1TU, 15TU and 27TU.

Fig. 4 demonstrates the binding of 1TU and 27TU to TNF-R1-DD. MBP, MBP-1TU or MBP-27TU (3μg) was incubated with glutathione beads containing 3μg of either GST or GST-TNF-R1-DD in 100μl of binding buffer (0.2%

Triton, 20 mM Tris pH 7.5, 140 mM NaCl, 0.1 mM EDTA, 10 mM DTT and 5% glycerol). The reaction ws performed at 4°C for 2 hours and centrifuged to remove unbound fraction (Unbound). The beads were then washed with 500µl binding buffer four times and resuspended into SDS-sample buffer (Bound). These samples were analyzed by Western blot using anti-MBP antibody (New England Biolab).

Fig. 5 demonstrates the ability of 15TU and 27TU to activate the JNK pathway. COS cells were contransfected with HA-tagged JNK1 and clones 15tu or 27TU. Cells were left untreated or treated for 15 min with 50 ng/ml TNF, and HA-JNK1 was immunoprecipitated with anti-HA antibody. JNK activity was measured in an *in vitro* kinase assay using GST-c-jun (amino acids 1-79) as substrate. and reactions were electrophoresed on SDS-PAGE.

Fig. 6 is an autoradiograph of an SDS-PAGE gel of conditioned media from COS cells transfected with clone 3TW.

Fig. 7 is an autoradiograph which demonstrates that an antisense oligonucleotide derived from the sequence of clone 3TW inhibits TNF-induced $cPLA_2$ phosphorylation.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have for the first time identified and isolated novel polynucleotides which encode proteins which bind to the TNF-R death domain. As used herein "TNF-R" includes all receptors for tumor necrosis factor. The P55 type TNF-R is the preferred receptor for practicing the present invention.

The sequence of a polynucleotide encoding one such protein is set forth in SEQ ID NO:1 from nucleotides 2 to 1231. This polynucleotide has been identified as "clone 2DD" The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 2DD is set forth in SEQ ID NO:2. It is believed that clone 2DD is a partial cDNA clone of a longer full length coding sequence. However. as demonstrated herein the protein encoded by clone 2DD does bind the death domain of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined herein). Clone 2DD was deposited with the American Type Culture Collection on October 13. 1994 and given the accession number ATCC 69706.

The protein encoded by clone 2DD is 410 amino acids in length. No identical or closely related sequences were found using BLASTN/BLASTX or FASTA searches. Therefore, clone 2DD encodes a novel protein.

The sequence of a polynucleotide encoding one such protein is set forth in SEQ ID NO:3 from nucleotides 2 to 415. This polynucleotide has been identified as "clone 3DD". The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 3DD is set forth in SEQ ID NO:4. It is believed that clone 3DD is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 3DD does bind the death domain of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined herein). Clone 3DD was deposited with the American Type Culture Collection on October 13, 1994 and given the accession number ATCC 69705.

The protein encoded by clone 3DD is 138 amino acids. No identical or closely related sequences were found using BLASTN/BLASTX or FASTA searches. Therefore, clone 3DD encodes a novel protein.

A full-length clone corresponding to clone 3DD was also isolated and identified as "clone 3TW". The nucleotide sequence of clone 3TW is reported as SEQ ID NO:13. Nucleotides 3 to 2846 of SEQ ID NO:13 encode a TNF-R1-DD ligand protein, the amino acid sequence of which is reported as SEQ ID NO:14. Amino acids 811 to 948 of SEQ ID NO:14 correspond to amino acids 1 to 138 of SEQ ID NO:4 (clone 3DD). Clone 3TW was deposited with the American Type Culture Collection on September 26, 1995 and given the accession number ATCC _____.

The sequence of a polynucleotide encoding another such protein is set forth in SEQ ID NO:5 from nucleotides 2 to 559. This polynucleotide has been identified as "clone 20DD." The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 20DD is set forth in SEQ ID NO:6. It is believed that clone 20DD is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 20DD does bind the death domain of TNF-R (*i.e.*, has "TNF-R1-DD ligand protein activity" as defined herein). Clone 20DD was deposited with the American Type Culture Collection on October 13, 1994 and given the accession number ATCC 69704.

The protein encoded by clone 20DD is identical to amino acids 87 to 272 of insulin-like growth factor binding protein-5 ("IGFBP-5"), a sequence for which was disclosed in J. Biol. Chem. *266*:10646-10653 (1991) by Shimasaki *et al.*, which is incorporated herein by reference. The polynucleotide and amino acid sequences of IGFBP-5 are set forth in SEQ ID NO:7 and SEQ ID NO:8, respectively. Based upon the sequence identity between clone 20DD and IGFBP-5, IGFBP-5 and certain fragments thereof will exhibit TNF-R1-DD ligand binding activity (as defined herein).

The sequence of a polynucleotide encoding another such protein is set forth in SEQ ID NO:9 from nucleotides 2 to 931. This polynucleotide has been identified as "clone 1TU" The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 1TU is set forth in SEQ ID NO:10. It is believed that clone 1TU is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 1TU does bind the death domain of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined herein). Clone 1TU was deposited with the American Type Culture Collection on June 7, 1995 and given the accession number ATCC 69848.

The protein encoded by clone 1TU is 310 amino acids in length. No identical or closely related sequences were found using BLASTN/BLASTX or FASTA searches. Therefore, clone 1TU encodes a novel protein.

The sequence of a polynucleotide encoding another such protein is set forth in SEQ ID NO:11 from nucleotides 2 to 1822. This polynucleotide has been identified as "clone 27TU" The amino acid sequence of the TNF-R1-DD ligand protein encoded by clone 27TU is set forth in SEQ ID NO:12. It is believed that clone 27TU is a partial cDNA clone of a longer full length coding sequence. However, as demonstrated herein the protein encoded by clone 27TU does bind the death domain of TNF-R (i.e., has "TNF-R1-DD ligand protein activity" as defined herein). Clone 27TU was deposited with the American Type Culture Collection on June 7, 1995 and given the accession number ATCC 69846.

The protein encoded by clone 27TU is 607 amino acids in length. No identical or closely related sequences were found using BLASTN/BLASTX or FASTA searches. Therefore, clone 27TU encodes a novel protein. 27TU may be

a longer version of clone 2DD. 2DD encodes the same amino acid sequence (SEQ ID NO:2) as amino acids 198-607 encoded by 27TU (SEQ ID NO:12). The nucleotide sequences of 2DD and 27TU are also identical within this region of identity.

An additional "clone 15TU" was isolated which encoded a portion of the 27TU sequence (approximately amino acids 289-607 of SEQ ID NO:12). Clone 15TU was deposited with the American Type Culture Collection on June 7, 1995 and given the accession number ATCC 69847. 15TU comprises the same nucleotide sequence as 27TU over this region of amino acids.

Polynucleotides hybridizing to the polynucleotides of the present invention under stringent conditions and highly stringent conditions are also part of the present invention. As used herein, "highly stringent conditions" include, for example, 0.2xSSC at 65°C; and "stringent conditions" include, for example, 4xSSC at 65°C or 50% formamide and 4xSSC at 42°C.

For the purposes of the present application, "TNF-R1-DD ligand protein" includes proteins which exhibit TNF-R1-DD ligand protein activity. For the purposes of the present application, a protein is defined as having "TNF-R1-DD ligand protein activity" when it binds to a protein derived from the TNF-R death domain. Activity can be measured by using any assay which will detect binding to an TNF-R death domain protein. Examples of such assays include without limitation the interaction trap assays and assays in which TNF-R death domain protein which is affixed to a surface in a manner conducive to observing binding, including without limitation those described in Examples 1 and 3. As used herein an "TNF-R death domain protein" includes the entire death domain or fragments thereof.

Fragments of the TNF-R1-DD ligand protein which are capable of interacting with the TNF-R death domain or which are capable of inhibiting TNF-R death domain binding (i.e., exhibit TNF-R1-DD ligand protein activity) are also encompassed by the present invention. Fragments of the TNF-R1-DD ligand protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules

such as immunoglobulins for many purposes, including increasing the valency of TNF-R1-DD ligand protein binding sites. For example, fragments of the TNF-R1-DD ligand protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the TNF-R1-DD ligand protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, an TNF-R1-DD ligand protein - IgM fusion would generate a decavalent form of the TNF-R1-DD ligand protein of the invention.

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the TNF-R1-DD ligand protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and the expression control sequence are situated within a vector or cell in such a way that the TNF-R1-DD ligand protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the TNF-R1-DD ligand protein. Host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

The TNF-R1-DD ligand protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described

in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference.

Alternatively, it may be possible to produce the TNF-R1-DD ligand protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida,* or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium,* or any bacterial strain capable of expressing heterologous proteins. If the TNF-R1-DD ligand protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional TNF-R1-DD ligand protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The TNF-R1-DD ligand protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the TNF-R1-DD ligand protein.

The TNF-R1-DD ligand protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the TNF-R1-DD ligand protein may also include an affinity column containing the TNF-R death domain or other TNF-R death domain protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the TNF-R1-DD ligand protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP) or glutathione-S-transferase (GST). Kits for expression and purification of such fusion

proteins are commercially available from New England BioLab (Beverly, MA) and Pharmacia (Piscataway, NJ), respectively. The TNF-R ligand protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the TNF-R1-DD ligand protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The TNF-R1-DD ligand protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated TNF-R1-DD ligand protein."

TNF-R1-DD ligand proteins may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with TNF-R1-DD ligand proteins may possess biological properties in common therewith, including TNF-R1-DD ligand protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified TNF-R1-DD ligand proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The TNF-R1-DD ligand proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified TNF-R1-DD ligand proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the TNF-R1-DD ligand protein sequences may include the replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Mutagenic techniques for such

replacement, insertion or deletion are well known to those skilled in the art (see. e.g., U.S. Patent No. 4,518,584).

Other fragments and derivatives of the sequences of TNF-R1-DD ligand proteins which would be expected to retain TNF-R1-DD ligand protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

TNF-R1-DD ligand protein of the invention may also be used to screen for agents which are capable of inhibiting or blocking binding of an TNF-R1-DD ligand protein to the death-domain of TNF-R, and thus may act as inhibitors of TNF-R death domain binding and/or TNF activity. Binding assays using a desired binding protein, immobilized or not, are well known in the art and may be used for this purpose using the TNF-R1-DD ligand protein of the invention. Examples 1 and 3 describe examples of such assays. Appropriate screening assays may be cell-based or cell-free. Alternatively, purified protein based screening assays may be used to identify such agents. For example, TNF-R1-DD ligand protein may be immobilized in purified form on a carrier and binding to purified TNF-R death domain may be measured in the presence and in the absence of potential inhibiting agents. A suitable binding assay may alternatively employ purified TNF-R death domain immobilized on a carrier, with a soluble form of a TNF-R1-DD ligand protein of the invention. Any TNF-R1-DD ligand protein may be used in the screening assays described above.

In such a screening assay, a first binding mixture is formed by combining TNF-R death domain protein and TNF-R1-DD ligand protein, and the amount of binding in the first binding mixture (B_o) is measured. A second binding mixture is also formed by combining TNF-R death domain protein, TNF-R1-DD ligand protein, and the compound or agent to be screened, and the amount of binding in the second binding mixture (B) is measured. The amounts of binding in the first and second binding mixtures are compared, for example, by performing a B/B_o calculation. A compound or agent is considered to be capable of inhibiting TNF-R death domain binding if a decrease in binding in the second binding mixture as

compared to the first binding mixture is observed. The formulation and optimization of binding mixtures is within the level of skill in the art. Such binding mixtures may also contain buffers and salts necessary to enhance or to optimize binding, and additional control assays may be included in the screening assay of the invention.

Alternatively, appropriate screening assays may be cell based. For example, the binding or interaction between an TNF-R ligand protein and the TNF-R death domain can be measured in yeast as described below in Examples 1 and 3.

Compounds found to reduce, preferably by at least about 10%, more preferably greater than about 50% or more, the binding activity of TNF-R1-DD ligand protein to TNF-R death domain may thus be identified and then secondarily screened in other binding assays, including *in-vivo* assays. By these means compounds having inhibitory activity for TNF-R death domain binding which may be suitable as anti-inflammatory agents may be identified.

Isolated TNF-R1-DD ligand protein may be useful in treating, preventing or ameliorating inflammatory conditions and other conditions, such as cachexia, autoimmune disease, graft versus host reaction, osteoporosis, colitis, myelogenous leukemia, diabetes, wasting, and atherosclerosis. Isolated TNF-R1-DD ligand protein may be used itself as an inhibitor of TNF-R death domain binding or to design inhibitors of TNF-R death domain binding. Inhibitors of binding of TNF-R1-DD ligand protein to the TNF-R death domain ("TNF-R intracellular binding inhibitors") are also useful for treating such conditions.

The present invention encompasses both pharmaceutical compositions and therapeutic methods of treatment or use which employ isolated TNF-R1-DD ligand protein and/or binding inhibitors of TNF-R intracellular binding.

Isolated TNF-R1-DD ligand protein or binding inhibitors (from whatever source derived, including without limitation from recombinant and non-recombinant cell lines) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to TNF-R1-DD ligand protein or binding inhibitor and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active

ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, G-CSF, Meg-CSF, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with isolated TNF-R1-DD ligand protein or binding inhibitor, or to minimize side effects caused by the isolated TNF-R1-DD ligand protein or binding inhibitor. Conversely, isolated TNF-R1-DD ligand protein or binding inhibitor may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which isolated TNF-R1-DD ligand protein or binding inhibitor is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of an inflammatory response or condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined

amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of isolated TNF-R1-DD ligand protein or binding inhibitor is administered to a mammal having a condition to be treated. Isolated TNF-R1-DD ligand protein or binding inhibitor may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, isolated TNF-R1-DD ligand protein or binding inhibitor may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering isolated TNF-R1-DD ligand protein or binding inhibitor in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of isolated TNF-R1-DD ligand protein or binding inhibitor used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of isolated TNF-R1-DD ligand protein or binding inhibitor is administered orally, isolated TNF-R1-DD ligand protein or binding inhibitor will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% isolated TNF-R1-DD ligand protein or binding inhibitor, and preferably from about 25 to 90% isolated TNF-R1-DD ligand protein or binding inhibitor. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological

saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of isolated TNF-R1-DD ligand protein or binding inhibitor, and preferably from about 1 to 50% isolated TNF-R1-DD ligand protein or binding inhibitor.

When a therapeutically effective amount of isolated TNF-R1-DD ligand protein or binding inhibitor is administered by intravenous, cutaneous or subcutaneous injection, isolated TNF-R1-DD ligand protein or binding inhibitor will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to isolated TNF-R1-DD ligand protein or binding inhibitor, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of isolated TNF-R1-DD ligand protein or binding inhibitor in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of isolated TNF-R1-DD ligand protein or binding inhibitor with which to treat each individual patient. Initially, the attending physician will administer low doses of isolated TNF-R1-DD ligand protein or binding inhibitor and observe the patient's response. Larger doses of isolated TNF-R1-DD ligand protein or binding inhibitor may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 µg to about 100 mg of isolated TNF-R1-DD ligand protein or binding inhibitor per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the isolated TNF-R1-DD ligand protein or binding inhibitor will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Isolated TNF-R1-DD ligand protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the TNF-R1-DD ligand protein and which may inhibit TNF-R death domain binding. Such antibodies may be obtained using either the entire TNF-R1-DD ligand protein or fragments of TNF-R1-DD ligand protein as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987).

Monoclonal antibodies binding to TNF-R1-DD ligand protein or to complex carbohydrate moieties characteristic of the TNF-R1-DD ligand glycoprotein may be useful diagnostic agents for the immunodetection of TNF-R ligand protein.

Neutralizing monoclonal antibodies binding to TNF-R1-DD ligand protein or to complex carbohydrates characteristic of TNF-R1-DD ligand glycoprotein may also be useful therapeutics for both inflammatory conditions and also in the treatment of some forms of cancer where abnormal expression of TNF-R1-DD ligand protein is involved. These neutralizing monoclonal antibodies are capable of blocking the signaling function of the TNF-R1-DD ligand protein. By blocking the binding of TNF-R1-DD ligand protein, certain biological responses to TNF are either abolished or markedly reduced. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against TNF-R1-DD ligand protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the TNF-R1-DD ligand protein.

Due to the similarity of their sequences to the insulin growth factor binding protein ("IGFBP-5") and fragments thereof which bind to the TNF-R death domain are proteins having TNF-R1-DD ligand protein activity as defined herein. As a result, they are also useful in pharmaceutical compositions, for treating inflammatory conditions and for inhibiting TNF-R death domain binding as described above for TNF-R1-DD ligand proteins generally.

EXAMPLE 1
CLONING OF TNF-R DEATH DOMAIN LIGAND PROTEIN ENCODING POLYNUCLEOTIDE

A yeast genetic selection method, the "interaction trap" [Gyuris et al, Cell 75:791-803, 1993, which is incorporated herein by reference], was used to screen WI38 cell cDNA libraries (preparation, see below) for proteins that interact with the death domain of the P55 type 1 TNF receptor (TNF-R1-DD). A polynucleotide encoding amino acids 326 to 413 of the P55 type TNF receptor, TNF-R1-DD, was obtained via the polymerase chain reaction (PCR) using a grafting method. This TNF-R1-DD DNA was then cloned into pEG202 by BamHI and SalI sites, generating the bait plasmid, pEG202-TNF-R1-DD. This plasmid contains the HIS3 selectable marker, and expression of the bait, the LexA-TNF-R1-DD fusion protein, is from the strong constitutive ADH1 promoter. To create the reporter strain carrying the bait protein, yeast strain EGY48, containing the reporter sequence LexAop-Leu2 in place of the chromosomal LEU2, was transformed with pEG202-TNF-R1-DD and pSH18-34 (Ura+), which carries another reporter sequence, LexAop-lacZ. For screening cDNAs encoding proteins that interact with TNF-R1-DD, the expression vector pJG4-5 (TRP1), containing the WI38 cell cDNA library (see below for the cDNA library construction), was transformed into the above strain (EGY48/pEG202-TNF-R1-DD/pSH18-34) according to the method described by Gietz *et al.*, Nucleic Acids Res., *20:*1425 (1992).

cDNA Library Construction:

WI38 cell cDNA library: Double stranded cDNA was prepared from 3ug of WI38 mRNA using reagents provided by the Superscript Choice System

(Gibco/BRL, Gaithersberg, MD) with the following substitutions: the first strand synthesis was primed using an oligo dT/XhoI primer/linker, and the dNTP mix was substituted with a mix containing methyl dCTP (Stratagene, LaJolla, CA). The cDNA was modified at both ends by addition of an EcoRI/NotI/SalI adapter linker and subsequently digested with XhoI. This produced cDNA molecules possessing an EcoRI/NotI/SalI overhang at the 5' end of the gene and an XhoI overhang at the 3' end. These fragments were then ligated into the yeast expression/fusion vector pJG4-5 (Gyuris et al., Cell, 75, 791-803, 1993), which contains at its amino terminus, the influenza virus HA1 epitope tag, the B42 acidic transcription activation domain, and the SV40 nuclear localization signal, all under the control of the galactose-dependent GAL1 promoter. The resulting plasmids were then electroporated into DH10B cells (Gibco/BRL). A total of 7.1 x 10^6 colonies were plated on LB plates containing 100 ug/ml of ampicillin. These *E.coli* were scraped, pooled, and a large scale plasmid prep was performed using the Wizard Maxi Prep kit (Promega, Madison, WI), yielding 3.2mg of supercoiled plasmid DNA.

WI38 Cell cDNA Screening Results:

1 x 10^6 transformants were obtained on glucose $Ura^-His^-Trp^-$ plates. These transformants were pooled and resuspended in a solution of 65% glycerol, 10mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$ and stored at -80°C in 1mL aliquots. For screening purposes, aliquots of these were diluted 10-fold into $Ura^-His^-Trp^-$ CM dropout gal/raff medium (containing 2% galactose, 1% raffinose), which induces the expresssion of the library encoded proteins, and incubated at 30°C for 4 hours. 12 x 10^6 colony forming units (CFUs) were then plated on standard 10cm galactose X-Gal $Ura^-His^-Trp^-Leu^-$ plates at a density of 2 x 10^5 CFU/plate. After three days at 30°C, about 1,000 colonies were formed (Leu^+) and of those, sixty-four colonies were $LacZ^+$. In order to test if the $Leu^+/LacZ^+$ phenotype was due to the library-encoded protein, the galactose dependency of the phenotype was tested. Expression of the library-encoded proteins was turned off by growth on glucose $Ura^-His^-Trp^-$ master plates and then retested for galactose-dependency on glucose $Ura^-His^-Trp^-Leu^+$, galactose $Ura^-His^-Trp^-Leu^+$, glucose X-Gal $Ura^-His^-Trp^+$, and galactose X-Gal $Ura^-His^-Trp^+$ plates. Of these, 32 colonies showed galactose-dependent growth on

Leu⁻ plates and galactose-dependent blue color on X-Gal-containing medium (LacZ⁻ phenotype). Total yeast DNA was prepared from these colonies according to the method described previously (Hoffman and Winston, 1987). In order to analyze the cDNA sequences, PCR reactions were performed using the above yeast DNA as a template and oligo primers specific for the vector pJG4-5, flanking the cDNA insertion point. PCR products were purified (Qiagen PCR purification kit), subjected to restriction digest with the enzyme HaeIII, run on 1.8% agarose gels, and the restriction patterns compared. Similar and identical restriction patterns were grouped and representatives of each group were sequenced and compared to Genbank and other databases to identify any sequence homologies.

One clone of unique sequence ("2DD") and three clones with identical sequence ("3DD") were isolated and showed no signficant sequence homologies compared to Genbank and other databases. Additionally, four other clones ("20DD") with identical sequence to a portion of human insulin-like growth factor binding protein-5 (Shunichi Shimasaki *et al.*, J. Biol. Chem. *266*:10646-10653 (1991)) were isolated. The clones "2DD," "3DD" and "20DD" were chosen for further analysis. Library vector pJG4-5 containing these clones sequences were rescued from yeast by transforming the total yeast DNAs into the *E. coli* strain KC8 and selecting for growth on Trp-ampicillin plates. These putative TNFR1 interacting proteins were then tested further for specificity of interaction with the TNF-R1-DD by the reintroduction of JG4-5 clone into EGY48 derivatives containing a panel of different baits, including bicoid, the cytoplasmic domain of the IL-1 receptor, and TNF-R1-DD. The above clones were found to interact only with the TNF-R1-DD. The interaction between these clones and TNF-R1-DD was thus judged to be specific.

U937 cDNA Screening Results:

A U937 cDNA library was also constructed and screened as described above. 1,020 Leu+ colonies were found and of those, 326 colonies were also LacZ+. 62 colonies of these Leu+/LacZ+ colonies showed a galactose-dependent phenotype.. One of these clones, 1TU, encodes a novel sequence. Interestingly, two clones, 15TU and 27TU, encode related or identical sequences, except that 27TU contains

about 864 additional nucleotides (or about 288 amino acids) at the 5' end. 15/27TU also encode a novel sequence.

EXAMPLE 2
EXPRESSION OF THE TNF-R1-DD ligand PROTEIN

cDNAs encoding TNF-R intracellular ligand proteins were released from the pJG4-5 vector with the appropriate restriction enzymes. For example, EcoRI and XhoI or NotI and XhoI were used to release cDNA from clone 2DD and clone 20DD. Where the restriction sites were also present in the internal sequence of the cDNA, PCR was performed to obtain the cDNA. For example, the cDNA fragment encoding "clone 3DD" was obtained through PCR due to the presence of an internal XhoI site. These cDNAs were then cloned into various expression vectors. These included pGEX (Pharmacia) or pMAL (New England Biolabs) for expression as a GST (Glutathione-S-transferase) or MBP (maltose binding protein) fusion protein in E. coli, a pED-based vector for mammalian expression, and pVL or pBlueBacHis (Invitrogen) for baculovirus/insect expression. For the immunodetection of TNF-R intracellular ligand expression in mammalian cells, an epitope sequence, "Flag," was inserted into the translational start site of the pED vector, generating the pED-Flag vector. cDNAs were then inserted into the pED-Flag vector. Thus, the expression of cDNA from pED-Flag yields a protein with an amino terminal Met, followed by the "Flag" sequence. Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys. Standard DEAE-Dextran or lipofectamine methods were used to transfect COS or CHO dukx cells. Immunodetection of Flag-tagged proteins was achieved using the M2 antibody (Kodak). Moreover, an immunoaffinity column using the M2 antibody, followed by elution with the "Flag" peptide, can be used for the rapid purification of the flag-tagged protein. Similarly, affinity purification of GST- , MBP- or His-tagged fusion proteins can be performed using glutathione, amylose, or nickel columns. Detailed purification protocols are provided by the manufacturers. For many fusion proteins, the TNF-R intracellular ligand can be released by the action of thrombin, factor Xa, or enterokinase cleavage. In the case where highly purified material is required, standard purification procedures, such as ion-exchange, hydrophobic, and

gel filtration chromatography will be applied in addition to the affinity purification step.

Figs. 1 and 2 depict autoradiographs demonstrating the expression of TNF-R1-DD ligand proteins in yeast and mammalian cells. Fig. 1 shows the results of expression of isloated clones of the present invention in yeast. EGY48 was transformed with pJG4-5 containing clone 2DD, 3DD or 20DD. Cells were then grown overnight in the galactose/raffinose medium. Cell lysates were prepared and subject to 4-20% SDS gel electrophoresis, followed by Western blot analysis using anti-HA antibody (12CA5, Boehringer Mannheim, Indianapolis, IN). Fig. 2 shows the results of expression of Flag-2DD and Flag-20DD in COS cells. COS cells were transfected with either pED-Flag (Vector control), Flag-2DD or Flag-20DD plasmid by the lipofectamine method. Thirty μg of each cell lysate were prepared and subjected to 4-20% SDS gel electrophoresis, followed by Western blot analysis using M2 antibody (Kodak). The bands in the Flag-2DD and Flag-20DD lanes indicate significant expression of the respective TNF-R1-DD ligand proteins.

EXAMPLE 3
ASSAYS OF TNF-R DEATH DOMAIN BINDING

Two different methods were used to assay for TNF-R1-DD ligand protein activity. The first assay measures binding in the yeast strain in "interaction trap." the system used here to screen for TNF-R1-DD interacting proteins. In this system, the expression of reporter genes from both LexAop-Leu2 and LexAop-LacZ relies on the interaction between the bait protein, in this case TNF-R1DD, and the prey, the TNF-R intracellular ligand. Thus, one can measure the strength of the interaction by the level of Leu2 or LacZ expression. The most simple method is to measure the activity of the LacZ encoded protein, β-galactosidase. This activity can be judged by the degree of blueness on the X-Gal containing medium or filter. For the quantitative measurement of β-galactosidase activity, standard assays can be found in "Methods in Yeast Genetics" Cold Spring Harbor, New York, 1990 (by Rose, M.D., Winston, F., and Hieter, P.).

The second assay for measuring binding is a cell-free system. An example of a typical assay is described below. Purified GST-TNF-R1-DD fusion

protein (2 ug) was mixed with amylose resins bound with a GST-TNF-R1-DD intracellular ligand for 2 hour at 4°C. The mixture was then centrifuged to separate bound (remained with the beads) and unbound (remained in the supernatant) GST-TNF-R1-DD. After extensive washing, the bound GST-TNF-R1-DD was eluted with maltose and detected by Western blot analysis using a GST antibody. The TNF-R1-DD or the intracellular ligand can also be immobilized on other solid supports, such as on plates or fluorobeads. The binding can then be measured using ELISA or SPA (scintillation proximity assay).

EXAMPLE 4
CHARACTERIZATION OF TNF-R DEATH DOMAIN LIGAND PROTEIN

Mapping the interaction site in TNF-R1

Many of the key amino acids for TNF-R signaling have been determined by site-directed mutagenesis (Tataglia *et al.*, Cell *74*:845-853 (1993). These amino acids are conserved between TNF-R and the Fas antigen, which is required for mediating cytotoxicity and other cellular responses. In order to test if the TNF-R intracellular proteins interact with these residues, the following mutations were constructed: F345A (substitution of phe at amino acid 345 to Ala), R347A, L351A, F345A/R347A/L351A, E369A, W378A and I408A. The ability of the mutant protein to interact with the intracellular ligand in the "interaction trap" system was tested.

Effect on the TNF-mediated response

The effect of the TNF-R intracellular ligands on the TNF-mediated response can be evaluated in cells overexpressing the ligands. A number of TNF-mediated responses, including transient or prolonged responses, can be measured. For example, TNF-induced kinase activity toward either MBP (myelin basic protein) or the N-terminus (amino acids 1-79) of c-jun can be measured in COS cells or CHO cells either transiently or stably overexpressing clone 2DD, 3DD or clone 20DD. The significance of these ligand proteins in TNF-mediated cytotoxicity and other cellular responses can be measured in L929 or U937 overexpressing cells.

Alternatively, other functional assays, such as the induction of gene expression or PGE_2 production after prolonged incubation with TNF, can also be used to measure the TNF mediated response. Conversely, the significance of the TNF-R1-DD ligand proteins in TNF signaling can be established by lowering or eliminating the expression of the ligands. These experiments can be performed using antisense expression or transgenic mice.

Enzymatic or functional assays

The signal transduction events initiated by TNF binding to its receptor are still largely unknown. However, one major result of TNF binding is the stimulation of cellular serine/threonine kinase activity. In addition, TNF has been shown to stimulate the activity of PC-PLC, PLA_2, and sphingomyelinase. Therefore, some of the TNF-R1-DD ligand proteins may possess intrinsic enzymatic activity that is responsible for these activities. Therefore, enzymatic assays can be performed to test this possibility, particularly with those clones that encode proteins with sequence homology to known enzymes. In addition to enzymatic activity, based on the sequence homology to proteins with known function, other functional assays can also be measured.

EXAMPLE 5
ISOLATION OF FULL LENGTH CLONES

In many cases, cDNAs obtained from the interaction trap method each encode only a portion of the full length protein. For example, based on identity and sequence and the lack of the initiating methionine codon, clones 2DD, 3DD and 20DD apparently do not encode full length proteins. Therefore, it is desirable to isolate full length clones. The cDNAs obtained from the screening, such as clone 2DD, are used as probes, and the cDNA libraries described herein, or alternatively phage cDNA libraries, are screened to obtain full length clones in accordance with known methods (see for example, "Molecular Cloning, A Laboratory Manual", by Sambrook et al., 1989 Cold Spring Harbor).

EXAMPLE 6
ANTIBODIES SPECIFIC FOR TNF-R INTRACELLULAR LIGAND PROTEIN

Antibodies specific for TNF-R intracellular ligand proteins can be produced using purified recombinant protein, as described in Example 2, as antigen. Both polyclonal and monoclonal antibodies will be produced using standard techniques, such as those described in "Antibodies, a Laboratory Manual" by Ed Harlow and David Lane (1988), Cold Spring Harbor Laboratory.

EXAMPLE 7
CHARACTERIZATION OF CLONES 1TU AND 15/27TU

Specificity of Interaction

The specificity of clones 1TU, 15TU and 27TU was tested using a panel of baits. The ability of these clones to bind the TNF-R death domain was compared to their binding to the intracellular domain of the second TNF-R (TNF-R $p75_{IC}$), the entire intracellular domain of TNF-R (TNF-R $p55_{IC}$), the death domain of the fas antigen (which shares 28% identity with TNF-R-DD) (Fas_{DD}), the *Drosophila* transcription factor bicoid, and a region of the IL-1 receptor known to be critical for signalling ($IL\text{-}1R_{477\text{-}527}$). As shown in Table 1, none of these clones interacted with TNF-R $p75_{IC}$ or Fas_{DD}, and only 1TU interacted with bicoid. In contrast, both 1TU and 15TU bound the cytoplasmic domain of the p55 TNF-R, as well as residues 477-527 of the IL-1R. 27TU interacted relatively weakly with these sequences.

Table 1

clone	$TNF\text{-}R_{DD}$	TNF-R $p75_{IC}$	TNF-R $p55_{IC}$	Fas_{DD}	bicoid	IL-1R (477-527)
1TU	+++	-	+++	-	++	+++
15TU	+++	±	+++	-	-	++
27TU	+++	-	+	-	-	+

Interaction with Amino Acids Critical for Signalling

The ability of each clone to interact with four single-site mutations in the TNF-R death domain (each known to abolish signalling) was measured. As shown in Table 2, each of the clones interacted less strongly with the death domain mutants than with the wild type death domain, suggesting that these clones may bind critical residues *in vivo*.

Table 2

clone	$TNF\text{-}R_{DD}$	F345A	L351A	W378A	I408A
1TU	+++	+	++	++	+
15TU	+++	+	+	++	++
27TU	+++	+	+	±	++

Expression of 1TU, 15TU and 27TU

Fig. 3 depicts an autoradiograph demonstrating the expression of clones 1TU, 15TU and 27TU in yeast (A) and COS cells (B).

In (A): EGY48 was transformed with pJG4-5 containing clones 1TU, 15TU or 27TU. Cells were then grown overnight in galactose/raffinose medium. Cell lysates were prepared and subjected to 4-20% SDS gel electrophoresis, followed by Western blot analysis using anti-HA antibody (12CA5, Boehringer Mannheim).

In (B): COS cells were transfected with pED-Flag containing clones 1TU, 15TU and 27TU. Cell lysates were prepared and analyzed by Western blot using anti-Flag antibody (M2, Kodak).

Specific Binding of 1TU and 27TU to TNF-R1-DD

The interaction of 1TU and 27TU with TNF-R1-DD was tested using purified bacterially expressed fusion proteins. As shown in Fig. 4, MBP fusion proteins containing 1TU or 27TU bound only to TNF-R1-DD expressed as a GST fusion protein, but not to GST protein alone. In the control experiment, MBP protein did not bind either GST or GST/TNF-R1-DD. These results indicate that

1TU and 27TU bound specifically to the TNF-R1 death domain *in vitro*, confirming the data obtained in the interaction trap.

15TU and 27TU Activation of JNK Activity

The jun N-terminal kinase (JNK) is normally activated within 15 min of TNF treatment in COS cells. 15TU and 27TU were cotransfected with an epitope tagged version of JNK, HA-JNK, in duplicate. After TNF treatment, JNK was immunoprecipitated with anti-HA antibody and JNK activity was measured in immunoprecipitation kinase assays, using GST-c-jun (amino acids 1-79) as substrate). Reactions were electrophoresed on SDS-PAGE. As shown in Fig. 5, transfection of 15TU and 27TU, but not vector alone, into COS cells activated JNK even in the absence of TNF, suggesting that these clones are involved in signal transduction of TNF and the pathway leading to JNK activation *in vivo*.

EXAMPLE 8
ISOLATION, EXPRESSION AND ASSAY OF CLONE 3TW

Clone 3TW was isolated from the WI38 cDNA library using clone 3DD as a porbe. Clone 3TW was expressed. Fig. 6 is an autoradiograph which demonstrates expression of 3TW (indicated by arrow).

An antisense oligonucleotide was derived from the sequence of clone 3TW. The antisense oligonucleotide was assayed to determine its ability to inhibit TNF-induced $cPLA_2$ phosphorylation. Fig. 7 depicts the results of that experiment. Activity of the anitsense oligonucleotide (3TWAS) was compared with the full-length clone (3TWFL), Flag-3TW full length (3TWFLflag) and pED-flag vector (pEDflag). The antisense oligonucleotide inhibited phosphorylation.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Lin, Lih-Ling
Chen, Jennifer H.
Schievella, Andrea
Graham, James

(ii) TITLE OF INVENTION: NOVEL TNF RECEPTOR DEATH DOMAIN LIGAND PROTEINS AND INHIBITORS OF LIGAND BINDING

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genetics Institute, Inc.
(B) STREET: 87 CambridgePark Drive
(C) CITY: Cambridge
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02140

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Brown, Scott A,
(B) REGISTRATION NUMBER: 32,724
(C) REFERENCE/DOCKET NUMBER: GI5232B

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 498-8224
(B) TELEFAX: (617) 876-5851

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2158 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..1231

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

C AGC AAT GCA GGT GAT GGA CCA GGT GGC GAG GGC AGT GTT CAC CTG
Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu Gly Ser Val His Leu

```
    1               5                  10                  15
GCA AGC TCT CGG GGC ACT TTG TCT GAT AGT GAA ATT GAG ACC AAC TCT          94
Ala Ser Ser Arg Gly Thr Leu Ser Asp Ser Glu Ile Glu Thr Asn Ser
            20                  25                  30

GCC ACA AGC ACC ATC TTT GGT AAA GCC CAC AGC TTG AAG CCA AGC ATA         142
Ala Thr Ser Thr Ile Phe Gly Lys Ala His Ser Leu Lys Pro Ser Ile
        35                  40                  45

AAG GAG AAG CTG GCA GGC AGC CCC ATT CGT ACT TCT GAA GAT GTG AGC         190
Lys Glu Lys Leu Ala Gly Ser Pro Ile Arg Thr Ser Glu Asp Val Ser
    50                  55                  60

CAG CGA GTC TAT CTC TAT GAG GGA CTC CTA GGC AAA GAG CGT TCT ACT         238
Gln Arg Val Tyr Leu Tyr Glu Gly Leu Leu Gly Lys Glu Arg Ser Thr
65                  70                  75

TTA TGG GAC CAA ATG CAA TTC TGG GAA GAT GCC TTC TTA GAT GCT GTG         286
Leu Trp Asp Gln Met Gln Phe Trp Glu Asp Ala Phe Leu Asp Ala Val
80                  85                  90                  95

ATG TTG GAG AGA GAA GGG ATG GGT ATG GAC CAG GGT CCC CAG GAA ATG         334
Met Leu Glu Arg Glu Gly Met Gly Met Asp Gln Gly Pro Gln Glu Met
                100                 105                 110

ATC GAC AGG TAC CTG TCC CTT GGA GAA CAT GAC CGG AAG CGC CTG GAA         382
Ile Asp Arg Tyr Leu Ser Leu Gly Glu His Asp Arg Lys Arg Leu Glu
            115                 120                 125

GAT GAT GAA GAT CGC TTG CTG GCC ACA CTT CTG CAC AAC CTC ATC TCC         430
Asp Asp Glu Asp Arg Leu Leu Ala Thr Leu Leu His Asn Leu Ile Ser
        130                 135                 140

TAC ATG CTG CTG ATG AAG GTA AAT AAG AAT GAC ATC CGC AAG AAG GTG         478
Tyr Met Leu Leu Met Lys Val Asn Lys Asn Asp Ile Arg Lys Lys Val
    145                 150                 155

AGG CGC CTA ATG GGA AAG TCG CAC ATT GGG CTT GTG TAC AGC CAG CAA         526
Arg Arg Leu Met Gly Lys Ser His Ile Gly Leu Val Tyr Ser Gln Gln
160                 165                 170                 175

ATC AAT GAG GTG CTT GAT CAG CTG GCG AAC CTG AAT GGA CGC GAT CTC         574
Ile Asn Glu Val Leu Asp Gln Leu Ala Asn Leu Asn Gly Arg Asp Leu
                180                 185                 190

TCT ATC TGG TCC AGT GGC AGC CGG CAC ATG AAG AAG CAG ACA TTT GTG         622
Ser Ile Trp Ser Ser Gly Ser Arg His Met Lys Lys Gln Thr Phe Val
            195                 200                 205

GTA CAT GCA GGG ACA GAT ACA AAC GGA GAT ATC TTT TTC ATG GAG GTG         670
Val His Ala Gly Thr Asp Thr Asn Gly Asp Ile Phe Phe Met Glu Val
        210                 215                 220

TGC GAT GAC TGT GTG GTG TTG CGT AGT AAC ATC GGA ACA GTG TAT GAG         718
Cys Asp Asp Cys Val Val Leu Arg Ser Asn Ile Gly Thr Val Tyr Glu
    225                 230                 235

CGC TGG TGG TAC GAG AAG CTC ATC AAC ATG ACC TAC TGT CCC AAG ACG         766
Arg Trp Trp Tyr Glu Lys Leu Ile Asn Met Thr Tyr Cys Pro Lys Thr
240                 245                 250                 255

AAG GTG TTG TGC TTG TGG CGT AGA AAT GGC TCT GAG ACC CAG CTC AAC         814
Lys Val Leu Cys Leu Trp Arg Arg Asn Gly Ser Glu Thr Gln Leu Asn
                260                 265                 270
```

```
AAG TTC TAT ACT AAA AAG TGT CGG GAG CTG TAC TAC TGT GTG AAG GAC        862
Lys Phe Tyr Thr Lys Lys Cys Arg Glu Leu Tyr Tyr Cys Val Lys Asp
            275                 280                 285

AGC ATG GAG CGC GCT GCC GCC CGA CAG CAA AGC ATC AAA CCC GGA CCT        910
Ser Met Glu Arg Ala Ala Ala Arg Gln Gln Ser Ile Lys Pro Gly Pro
        290                 295                 300

GAA TTG GGT GGC GAG TTC CCT GTG CAG GAC CTG AAG ACT GGT GAG GGT        958
Glu Leu Gly Gly Glu Phe Pro Val Gln Asp Leu Lys Thr Gly Glu Gly
    305                 310                 315

GGC CTG CTG CAG GTG ACC CTG GAA GGG ATC AAC CTC AAA TTC ATG CAC       1006
Gly Leu Leu Gln Val Thr Leu Glu Gly Ile Asn Leu Lys Phe Met His
320                 325                 330                 335

AAT CAG GTT TTC ATA GAG CTG AAT CAC ATT AAA AAG TGC AAT ACA GTT       1054
Asn Gln Val Phe Ile Glu Leu Asn His Ile Lys Lys Cys Asn Thr Val
                340                 345                 350

CGA GGC GTC TTT GTC CTG GAG GAA TTT GTT CCT GAA ATT AAA GAA GTG       1102
Arg Gly Val Phe Val Leu Glu Glu Phe Val Pro Glu Ile Lys Glu Val
            355                 360                 365

GTG AGC CAC AAG TAC AAG ACA CCA ATG GCC CAC GAA ATC TGC TAC TCC       1150
Val Ser His Lys Tyr Lys Thr Pro Met Ala His Glu Ile Cys Tyr Ser
        370                 375                 380

GTA TTA TGT CTC TTC TCG TAC GTG GCT GCA GTT CAT AGC AGT GAG GAA       1198
Val Leu Cys Leu Phe Ser Tyr Val Ala Ala Val His Ser Ser Glu Glu
    385                 390                 395

GAT CTC AGA ACC CCG CCC CGG CCT GTC TCT AGC TGATGGAGAG GGGCTACGCA     1251
Asp Leu Arg Thr Pro Pro Arg Pro Val Ser Ser
400                 405                 410

GCTGCCCCAG CCCAGGGCAC GCCCCTGGCC CCTTGCTGTT CCCAAGTGCA CGATGCTGCT     1311

GTGACTGAGG AGTGGATGAT GCTCGTGTGT CCTCTGCAAG CCCCCTGCTG TGGCTTGGGT     1371

GGGTACCGGT TATGTGTCCC TCTGAGTGTG TCTTGAGCGT GTCCACCTTC TCCCTCTCCA     1431

CTCCCAGAAG ACCAAACTGC CTTCCCCTCA GGGCTCAAGA ATGTGTACAG TCTGTGGGGC     1491

CGGTGTGAAC CCACTATTTT GTGTCCTTGA GACATTTGTG TTGTGGTTCC TTGTCCTTGT     1551

CCCTGGCGTT AACTGTCCAC TGCAAGAGTC TGGCTCTCCC TTCTCTGTGA CCCGGCATGA     1611

CTGGGCGCCT GGAGCAGTTT CACTCTGTGA GGAGTGAGGG AACCCTGGGG CTCACCCTCT     1671

CAGAGGAAGG GCACAGAGAG GAAGGGAAGA ATTGGGGGGC AGCCGGAGTG AGTGGCAGCC     1731

TCCCTGCTTC CTTCTGCATT CCCAAGCCGG CAGCTACTGC CCAGGGCCCG CAGTGTTGGC     1791

TGCTGCCTGC CACAGCCTCT GTGACTGCAG TGGAGCGGCG AATTCCCTGT GGCCTGCCAC     1851

GCCTTCGGCA TCAGAGGATG GAGTGGTCGA GGCTAGTGGA GTCCCAGGGA CCGCTGGCTG     1911

CTCTGCCTGA GCATCAGGGA GGGGGCAGGA AAGACCAAGC TGGGTTTGCA CATCTGTCTG     1971

CAGGCTGTCT CTCCAGGCAC GGGGTGTCAG GAGGGAGAGA CAGCCTGGGT ATGGGCAAGA     2031

AATGACTGTA AATATTTCAG CCCCACATTA TTTATAGAAA ATGTACAGTT GTGTGAATGT     2091

GAAATAAATG TCCTCACCTC CCAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA     2151

AAAAAAA                                                              2158
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 410 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu Gly Ser Val His Leu Ala
1 5 10 15

Ser Ser Arg Gly Thr Leu Ser Asp Ser Glu Ile Glu Thr Asn Ser Ala
20 25 30

Thr Ser Thr Ile Phe Gly Lys Ala His Ser Leu Lys Pro Ser Ile Lys
35 40 45

Glu Lys Leu Ala Gly Ser Pro Ile Arg Thr Ser Glu Asp Val Ser Gln
50 55 60

Arg Val Tyr Leu Tyr Glu Gly Leu Leu Gly Lys Glu Arg Ser Thr Leu
65 70 75 80

Trp Asp Gln Met Gln Phe Trp Glu Asp Ala Phe Leu Asp Ala Val Met
85 90 95

Leu Glu Arg Glu Gly Met Gly Met Asp Gln Gly Pro Gln Glu Met Ile
100 105 110

Asp Arg Tyr Leu Ser Leu Gly Glu His Asp Arg Lys Arg Leu Glu Asp
115 120 125

Asp Glu Asp Arg Leu Leu Ala Thr Leu Leu His Asn Leu Ile Ser Tyr
130 135 140

Met Leu Leu Met Lys Val Asn Lys Asn Asp Ile Arg Lys Lys Val Arg
145 150 155 160

Arg Leu Met Gly Lys Ser His Ile Gly Leu Val Tyr Ser Gln Gln Ile
165 170 175

Asn Glu Val Leu Asp Gln Leu Ala Asn Leu Asn Gly Arg Asp Leu Ser
180 185 190

Ile Trp Ser Ser Gly Ser Arg His Met Lys Lys Gln Thr Phe Val Val
195 200 205

His Ala Gly Thr Asp Thr Asn Gly Asp Ile Phe Phe Met Glu Val Cys
210 215 220

Asp Asp Cys Val Val Leu Arg Ser Asn Ile Gly Thr Val Tyr Glu Arg
225 230 235 240

Trp Trp Tyr Glu Lys Leu Ile Asn Met Thr Tyr Cys Pro Lys Thr Lys
245 250 255

Val Leu Cys Leu Trp Arg Arg Asn Gly Ser Glu Thr Gln Leu Asn Lys
260 265 270

Phe Tyr Thr Lys Lys Cys Arg Glu Leu Tyr Tyr Cys Val Lys Asp Ser
275 280 285

```
Met Glu Arg Ala Ala Ala Arg Gln Gln Ser Ile Lys Pro Gly Pro Glu
    290                 295                 300

Leu Gly Gly Glu Phe Pro Val Gln Asp Leu Lys Thr Gly Glu Gly Gly
305                 310                 315                 320

Leu Leu Gln Val Thr Leu Glu Gly Ile Asn Leu Lys Phe Met His Asn
                325                 330                 335

Gln Val Phe Ile Glu Leu Asn His Ile Lys Lys Cys Asn Thr Val Arg
            340                 345                 350

Gly Val Phe Val Leu Glu Glu Phe Val Pro Glu Ile Lys Glu Val Val
        355                 360                 365

Ser His Lys Tyr Lys Thr Pro Met Ala His Glu Ile Cys Tyr Ser Val
    370                 375                 380

Leu Cys Leu Phe Ser Tyr Val Ala Ala Val His Ser Ser Glu Glu Asp
385                 390                 395                 400

Leu Arg Thr Pro Pro Arg Pro Val Ser Ser
                405                 410
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 826 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..415

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
G GAG GTG CAG GAC CTC TTC GAA GCC CAG GGC AAT GAC CGA CTG AAG          46
  Glu Val Gln Asp Leu Phe Glu Ala Gln Gly Asn Asp Arg Leu Lys
    1               5                  10                  15

CTG CTG GTG CTG TAC AGT GGA GAG GAT GAT GAG CTG CTA CAG CGG GCA        94
Leu Leu Val Leu Tyr Ser Gly Glu Asp Asp Glu Leu Leu Gln Arg Ala
                20                  25                  30

GCT GCC GGG GGC TTG GCC ATG CTT ACC TCC ATG CGG CCC ACG CTC TGC       142
Ala Ala Gly Gly Leu Ala Met Leu Thr Ser Met Arg Pro Thr Leu Cys
            35                  40                  45

AGC CGC ATT CCC CAA GTG ACC ACA CAC TGG CTG GAG ATC CTG CAG GCC       190
Ser Arg Ile Pro Gln Val Thr Thr His Trp Leu Glu Ile Leu Gln Ala
        50                  55                  60

CTG CTT CTG AGC TCC AAC CAG GAG CTG CAG CAC CGG GGT GCT GTG GTG       238
Leu Leu Leu Ser Ser Asn Gln Glu Leu Gln His Arg Gly Ala Val Val
    65                  70                  75

GTG CTG AAC ATG GTG GAG GCC TCG AGG GAG ATT GCC AGC ACC CTG ATG       286
Val Leu Asn Met Val Glu Ala Ser Arg Glu Ile Ala Ser Thr Leu Met
80                  85                  90                  95
```

```
GAG AGT GAG ATG ATG GAG ATC TTG TCA GTG CTA GCT AAG GGT GAC CAC          334
Glu Ser Glu Met Met Glu Ile Leu Ser Val Leu Ala Lys Gly Asp His
                100                 105                 110

AGC CCT GTC ACA AGG GCT GCT GCA GCC TGC CTG GAC AAA GCA GTG GAA          382
Ser Pro Val Thr Arg Ala Ala Ala Ala Cys Leu Asp Lys Ala Val Glu
            115                 120                 125

TAT GGG CTT ATC CAA CCC AAC CAA GAT GGA GAG TGAGGGGGTT GTCCCTGGGC        435
Tyr Gly Leu Ile Gln Pro Asn Gln Asp Gly Glu
        130                 135

CCAAGGCTCA TGCACACGCT ACCTATTGTG GCACGGAGAG TAAGGACGGA AGCAGCTTTG        495

GCTGGTGGTG GCTGGCATGC CCAATACTCT TGCCCATCCT CGCTTGCTGC CCTAGGATGT        555

CCTCTGTTCT GAGTCAGCGG CCACGTTCAG TCACACAGCC CTGCTTGGCC AGCACTGCCT        615

GCAGCCTCAC TCAGAGGGGC CCTTTTTCTG TACTACTGTA GTCAGCTGGG AATGGGGAAG        675

GTGCATCCCA ACACAGCCTG TGGATCCTGG GGCATTTGGA AGGGCGCACA CATCAGCAGC        735

CTCACCAGCT GTGAGCCTGC TATCAGGCCT GCCCCTCCAA TAAAAGTGTG TAGAACTCCA        795

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A                                       826
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 138 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Glu Val Gln Asp Leu Phe Glu Ala Gln Gly Asn Asp Arg Leu Lys Leu
 1               5                  10                  15

Leu Val Leu Tyr Ser Gly Glu Asp Asp Glu Leu Leu Gln Arg Ala Ala
            20                  25                  30

Ala Gly Gly Leu Ala Met Leu Thr Ser Met Arg Pro Thr Leu Cys Ser
        35                  40                  45

Arg Ile Pro Gln Val Thr Thr His Trp Leu Glu Ile Leu Gln Ala Leu
    50                  55                  60

Leu Leu Ser Ser Asn Gln Glu Leu Gln His Arg Gly Ala Val Val Val
65                  70                  75                  80

Leu Asn Met Val Glu Ala Ser Arg Glu Ile Ala Ser Thr Leu Met Glu
                85                  90                  95

Ser Glu Met Met Glu Ile Leu Ser Val Leu Ala Lys Gly Asp His Ser
            100                 105                 110

Pro Val Thr Arg Ala Ala Ala Ala Cys Leu Asp Lys Ala Val Glu Tyr
        115                 120                 125

Gly Leu Ile Gln Pro Asn Gln Asp Gly Glu
    130                 135
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 722 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..559

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
G GAG AAG CCG CTG CAC GCC CTG CTG CAC GGC CGC GGG GTT TGC CTC          46
  Glu Lys Pro Leu His Ala Leu Leu His Gly Arg Gly Val Cys Leu
    1               5                  10                  15

AAC GAA AAG AGC TAC CGC GAG CAA GTC AAG ATC GAG AGA GAC TCC CGT        94
Asn Glu Lys Ser Tyr Arg Glu Gln Val Lys Ile Glu Arg Asp Ser Arg
                  20                  25                  30

GAG CAC GAG GAG CCC ACC ACC TCT GAG ATG GCC GAG GAG ACC TAC TCC       142
Glu His Glu Glu Pro Thr Thr Ser Glu Met Ala Glu Glu Thr Tyr Ser
             35                  40                  45

CCC AAG ATC TTC CGG CCC AAA CAC ACC CGC ATC TCC GAG CTG AAG GCT       190
Pro Lys Ile Phe Arg Pro Lys His Thr Arg Ile Ser Glu Leu Lys Ala
         50                  55                  60

GAA GCA GTG AAG AAG GAC CGC AGA AAG AAG CTG ACC CAG TCC AAG TTT       238
Glu Ala Val Lys Lys Asp Arg Arg Lys Lys Leu Thr Gln Ser Lys Phe
     65                  70                  75

GTC GGG GGA GCC GAG AAC ACT GCC CAC CCC CGG ATC ATC TCT GAA CCT       286
Val Gly Gly Ala Glu Asn Thr Ala His Pro Arg Ile Ile Ser Glu Pro
 80                  85                  90                  95

GAG ATG AGA CAG GAG TCT GAG CAG GGC CCC TGC CGC AGA CAC ATG GAG       334
Glu Met Arg Gln Glu Ser Glu Gln Gly Pro Cys Arg Arg His Met Glu
                100                 105                 110

GCT TCC CTG CAG GAG CTC AAA GCC AGC CCA CGC ATG GTG CCC CGT GCT       382
Ala Ser Leu Gln Glu Leu Lys Ala Ser Pro Arg Met Val Pro Arg Ala
            115                 120                 125

GTG TAC CTG CCC AAT TGT GAC CGC AAA GGA TTC TAC AAG AGA AAG CAG       430
Val Tyr Leu Pro Asn Cys Asp Arg Lys Gly Phe Tyr Lys Arg Lys Gln
        130                 135                 140

TGC AAA CCT TCC CGT GGC CGC AAG CGT GGC ATC TGC TGG TGC GTG GAC       478
Cys Lys Pro Ser Arg Gly Arg Lys Arg Gly Ile Cys Trp Cys Val Asp
    145                 150                 155

AAG TAC GGG ATG AAG CTG CCA GGC ATG GAG TAC GTT GAC GGG GAC TTT       526
Lys Tyr Gly Met Lys Leu Pro Gly Met Glu Tyr Val Asp Gly Asp Phe
160                 165                 170                 175

CAG TGC CAC ACC TTC GAC AGC AGC AAC GTT GAG TGATGCGTCC CCCCCCAACC      579
Gln Cys His Thr Phe Asp Ser Ser Asn Val Glu
                180                 185

TTTCCCTCAC CCCCTTCCAC CCCCAGCCCC GACTCCAGCC AGCGCCTCCC TCCACCCCAG      639
```

GACGCCACTC ATTTCATCTC ATTTAAGGGA AAAATATATA TCTATCTATT TGAGGAAAAA 699

AAAAAAAAAA AAAAAAAAAA AAA 722

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 186 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Glu Lys Pro Leu His Ala Leu Leu His Gly Arg Gly Val Cys Leu Asn
1 5 10 15

Glu Lys Ser Tyr Arg Glu Gln Val Lys Ile Glu Arg Asp Ser Arg Glu
20 25 30

His Glu Glu Pro Thr Thr Ser Glu Met Ala Glu Glu Thr Tyr Ser Pro
35 40 45

Lys Ile Phe Arg Pro Lys His Thr Arg Ile Ser Glu Leu Lys Ala Glu
50 55 60

Ala Val Lys Lys Asp Arg Arg Lys Lys Leu Thr Gln Ser Lys Phe Val
65 70 75 80

Gly Gly Ala Glu Asn Thr Ala His Pro Arg Ile Ile Ser Glu Pro Glu
85 90 95

Met Arg Gln Glu Ser Glu Gln Gly Pro Cys Arg Arg His Met Glu Ala
100 105 110

Ser Leu Gln Glu Leu Lys Ala Ser Pro Arg Met Val Pro Arg Ala Val
115 120 125

Tyr Leu Pro Asn Cys Asp Arg Lys Gly Phe Tyr Lys Arg Lys Gln Cys
130 135 140

Lys Pro Ser Arg Gly Arg Lys Arg Gly Ile Cys Trp Cys Val Asp Lys
145 150 155 160

Tyr Gly Met Lys Leu Pro Gly Met Glu Tyr Val Asp Gly Asp Phe Gln
165 170 175

Cys His Thr Phe Asp Ser Ser Asn Val Glu
180 185

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1023 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 57..875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CCCTGCACTC TCGCTCTCCT GCCCCACCCC GAGGTAAAGG GGGCGACTAA GAGAAG          56

ATG GTG TTG CTC ACC GCG GTC CTC CTG CTG CTG GCC GCC TAT GCG GGG       104
Met Val Leu Leu Thr Ala Val Leu Leu Leu Ala Ala Tyr Ala Gly
 1               5                  10                  15

CCG GCC CAG AGC CTG GGC TCC TTC GTG CAC TGC GAG CCC TGC GAC GAG       152
Pro Ala Gln Ser Leu Gly Ser Phe Val His Cys Glu Pro Cys Asp Glu
            20                  25                  30

AAA GCC CTC TCC ATG TGC CCC CCC AGC CCC CTG GGC TGC GAG CTG GTC       200
Lys Ala Leu Ser Met Cys Pro Pro Ser Pro Leu Gly Cys Glu Leu Val
        35                  40                  45

AAG GAG CCG GGC TGC GGC TGC TGC ATG ACC TGC GCC CTG GCC GAG GGG       248
Lys Glu Pro Gly Cys Gly Cys Cys Met Thr Cys Ala Leu Ala Glu Gly
    50                  55                  60

CAG TCG TGC GGC GTC TAC ACC GAG CGC TGC GCC CAG GGG CTG CGC TGC       296
Gln Ser Cys Gly Val Tyr Thr Glu Arg Cys Ala Gln Gly Leu Arg Cys
65                  70                  75                  80

CTC CCC CGG CAG GAC GAG GAG AAG CCG CTG CAC GCC CTG CTG CAC GGC       344
Leu Pro Arg Gln Asp Glu Glu Lys Pro Leu His Ala Leu Leu His Gly
                85                  90                  95

CGC GGG GTT TGC CTC AAC GAA AAG AGC TAC CGC GAG CAA GTC AAG ATC       392
Arg Gly Val Cys Leu Asn Glu Lys Ser Tyr Arg Glu Gln Val Lys Ile
            100                 105                 110

GAG AGA GAC TCC CGT GAG CAC GAG GAG CCC ACC ACC TCT GAG ATG GCC       440
Glu Arg Asp Ser Arg Glu His Glu Glu Pro Thr Thr Ser Glu Met Ala
        115                 120                 125

GAG GAG ACC TAC TCC CCC AAG ATC TTC CGG CCC AAA CAC ACC CGC ATC       488
Glu Glu Thr Tyr Ser Pro Lys Ile Phe Arg Pro Lys His Thr Arg Ile
    130                 135                 140

TCC GAG CTG AAG GCT GAA GCA GTG AAG AAG GAC CGC AGA AAG AAG CTG       536
Ser Glu Leu Lys Ala Glu Ala Val Lys Lys Asp Arg Arg Lys Lys Leu
145                 150                 155                 160

ACC CAG TCC AAG TTT GTC GGG GGA GCC GAG AAC ACT GCC CAC CCC CGG       584
Thr Gln Ser Lys Phe Val Gly Gly Ala Glu Asn Thr Ala His Pro Arg
                165                 170                 175

ATC ATC TCT GCA CCT GAG ATG AGA CAG GAG TCT GAG CAG GGC CCC TGC       632
Ile Ile Ser Ala Pro Glu Met Arg Gln Glu Ser Glu Gln Gly Pro Cys
            180                 185                 190

CGC AGA CAC ATG GAG GCT TCC CTG CAG GAG CTC AAA GCC AGC CCA CGC       680
Arg Arg His Met Glu Ala Ser Leu Gln Glu Leu Lys Ala Ser Pro Arg
        195                 200                 205

ATG GTG CCC CGT GCT GTG TAC CTG CCC AAT TGT GAC CGC AAA GGA TTC       728
Met Val Pro Arg Ala Val Tyr Leu Pro Asn Cys Asp Arg Lys Gly Phe
    210                 215                 220

TAC AAG AGA AAG CAG TGC AAA CCT TCC CGT GGC CGC AAG CGT GGC ATC       776
Tyr Lys Arg Lys Gln Cys Lys Pro Ser Arg Gly Arg Lys Arg Gly Ile
225                 230                 235                 240
```

```
TGC TGG TGC GTG GAC AAG TAC GGG ATG AAG CTG CCA GGC ATG GAG TAC          824
Cys Trp Cys Val Asp Lys Tyr Gly Met Lys Leu Pro Gly Met Glu Tyr
                245                 250                 255

GTT GAC GGG GAC TTT CAG TGC CAC ACC TTC GAC AGC AGC AAC GTT GAG          872
Val Asp Gly Asp Phe Gln Cys His Thr Phe Asp Ser Ser Asn Val Glu
            260                 265                 270

TGATGCGTCC CCCCCCAACC TTTCCCTCAC CCCCTCCCAC CCCCAGCCCC GACTCCAGCC        932

AGCGCCTCCC TCCACCCCAG GACGCCACTC ATTTCATCTC ATTTAAGGGA AAAATATATA        992

TCTATCTATT TGAAAAAAAA AAAAAAAACC C                                      1023
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 272 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Val Leu Leu Thr Ala Val Leu Leu Leu Leu Ala Ala Tyr Ala Gly
 1               5                  10                  15

Pro Ala Gln Ser Leu Gly Ser Phe Val His Cys Glu Pro Cys Asp Glu
            20                  25                  30

Lys Ala Leu Ser Met Cys Pro Pro Ser Pro Leu Gly Cys Glu Leu Val
        35                  40                  45

Lys Glu Pro Gly Cys Gly Cys Cys Met Thr Cys Ala Leu Ala Glu Gly
    50                  55                  60

Gln Ser Cys Gly Val Tyr Thr Glu Arg Cys Ala Gln Gly Leu Arg Cys
65                  70                  75                  80

Leu Pro Arg Gln Asp Glu Glu Lys Pro Leu His Ala Leu Leu His Gly
                85                  90                  95

Arg Gly Val Cys Leu Asn Glu Lys Ser Tyr Arg Glu Gln Val Lys Ile
            100                 105                 110

Glu Arg Asp Ser Arg Glu His Glu Glu Pro Thr Thr Ser Glu Met Ala
        115                 120                 125

Glu Glu Thr Tyr Ser Pro Lys Ile Phe Arg Pro Lys His Thr Arg Ile
    130                 135                 140

Ser Glu Leu Lys Ala Glu Ala Val Lys Lys Asp Arg Arg Lys Lys Leu
145                 150                 155                 160

Thr Gln Ser Lys Phe Val Gly Gly Ala Glu Asn Thr Ala His Pro Arg
                165                 170                 175

Ile Ile Ser Ala Pro Glu Met Arg Gln Glu Ser Glu Gln Gly Pro Cys
            180                 185                 190

Arg Arg His Met Glu Ala Ser Leu Gln Glu Leu Lys Ala Ser Pro Arg
        195                 200                 205

Met Val Pro Arg Ala Val Tyr Leu Pro Asn Cys Asp Arg Lys Gly Phe
    210                 215                 220
```

```
Tyr Lys Arg Lys Gln Cys Lys Pro Ser Arg Gly Arg Lys Arg Gly Ile
225                 230                 235                 240

Cys Trp Cys Val Asp Lys Tyr Gly Met Lys Leu Pro Gly Met Glu Tyr
                245                 250                 255

Val Asp Gly Asp Phe Gln Cys His Thr Phe Asp Ser Ser Asn Val Glu
            260                 265                 270
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1694 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..931

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
C TCT CTC AAG GCC AAC ATC CCT GAG GTG GAA GCT GTC CTC AAC ACC          46
  Ser Leu Lys Ala Asn Ile Pro Glu Val Glu Ala Val Leu Asn Thr
    1               5                  10                  15

GAC AGG AGT TTG GTG TGT GAT GGG AAG AGG GGC TTA TTA ACT CGT CTG        94
Asp Arg Ser Leu Val Cys Asp Gly Lys Arg Gly Leu Leu Thr Arg Leu
                20                  25                  30

CTG CAG GTC ATG AAG AAG GAG CCA GCA GAG TCG TCT TTC AGG TTT TGG       142
Leu Gln Val Met Lys Lys Glu Pro Ala Glu Ser Ser Phe Arg Phe Trp
            35                  40                  45

CAA GCT CGG GCT GTG GAG AGT TTC CTC CGA GGG ACC ACC TCC TAT GCA       190
Gln Ala Arg Ala Val Glu Ser Phe Leu Arg Gly Thr Thr Ser Tyr Ala
        50                  55                  60

GAC CAG ATG TTC CTG CTG AAG CGA GGC CTC TTG GAG CAC ATC CTT TAC       238
Asp Gln Met Phe Leu Leu Lys Arg Gly Leu Leu Glu His Ile Leu Tyr
    65                  70                  75

TGC ATT GTG GAC AGC GAG TGT AAG TCA AGG GAT GTG CTC CAG AGT TAC       286
Cys Ile Val Asp Ser Glu Cys Lys Ser Arg Asp Val Leu Gln Ser Tyr
80                  85                  90                  95

TTT GAC CTC CTG GGG GAG CTG ATG AAG TTC AAC GTT GAT GCA TTC AAG       334
Phe Asp Leu Leu Gly Glu Leu Met Lys Phe Asn Val Asp Ala Phe Lys
                100                 105                 110

AGA TTC AAT AAA TAT ATC AAC ACC GAT GCA AAG TTC CAG GTA TTC CTG       382
Arg Phe Asn Lys Tyr Ile Asn Thr Asp Ala Lys Phe Gln Val Phe Leu
            115                 120                 125
```

```
AAG CAG ATC AAC AGC TCC CTG GTG GAC TCC AAC ATG CTG GTG CGC TGT          430
Lys Gln Ile Asn Ser Ser Leu Val Asp Ser Asn Met Leu Val Arg Cys
        130                 135                 140

GTC ACT CTG TCC CTG GAC CGA TTT GAA AAC CAG GTG GAT ATG AAA GTT          478
Val Thr Leu Ser Leu Asp Arg Phe Glu Asn Gln Val Asp Met Lys Val
    145                 150                 155

GCC GAG GTA CTG TCT GAA TGC CGC CTG CTC GCC TAC ATA TCC CAG GTG          526
Ala Glu Val Leu Ser Glu Cys Arg Leu Leu Ala Tyr Ile Ser Gln Val
160                 165                 170                 175

CCC ACG CAG ATG TCC TTC CTC TTC CGC CTC ATC AAC ATC ATC CAC GTG          574
Pro Thr Gln Met Ser Phe Leu Phe Arg Leu Ile Asn Ile Ile His Val
                180                 185                 190

CAG ACG CTG ACC CAG GAG AAC GTC AGC TGC CTC AAC ACC AGC CTG GTG          622
Gln Thr Leu Thr Gln Glu Asn Val Ser Cys Leu Asn Thr Ser Leu Val
            195                 200                 205

ATC CTG ATG CTG GCC CGA CGG AAA GAG CGG CTG CCC CTG TAC CTG CGG          670
Ile Leu Met Leu Ala Arg Arg Lys Glu Arg Leu Pro Leu Tyr Leu Arg
        210                 215                 220

CTG CTG CAG CGG ATG GAG CAC AGC AAG AAG TAC CCC GGC TTC CTG CTC          718
Leu Leu Gln Arg Met Glu His Ser Lys Lys Tyr Pro Gly Phe Leu Leu
    225                 230                 235

AAC AAC TTC CAC AAC CTG CTG CGC TTC TGG CAG CAG CAC TAC CTG CAC          766
Asn Asn Phe His Asn Leu Leu Arg Phe Trp Gln Gln His Tyr Leu His
240                 245                 250                 255

AAG GAC AAG GAC AGC ACC TGC CTA GAG AAC AGC TCC TGC ATC AGC TTC          814
Lys Asp Lys Asp Ser Thr Cys Leu Glu Asn Ser Ser Cys Ile Ser Phe
                260                 265                 270

TCA TAC TGG AAG GAG ACA GTG TCC ATC CTG TTG AAC CCG GAC CGG CAG          862
Ser Tyr Trp Lys Glu Thr Val Ser Ile Leu Leu Asn Pro Asp Arg Gln
            275                 280                 285

TCA CCC TCT GCT CTC GTT AGC TAC ATT GAG GAG CCC TAC ATG GAC ATA          910
Ser Pro Ser Ala Leu Val Ser Tyr Ile Glu Glu Pro Tyr Met Asp Ile
        290                 295                 300

GAC AGG GAC TTC ACT GAG GAG TGACCTTGGG CCAGGCCTCG GGAGGCTGCT             961
Asp Arg Asp Phe Thr Glu Glu
    305                 310

GGGCCAGTGT GGGTGAGCGT GGGTACGATG CCACACGCCC TGCCCTGTTC CCGTTCCTCC       1021

CTGCTGCTCT CTGCCTGCCC CAGGTCTTTG GGTACAGGCT TGGTGGGAGG GAAGTCCTAG       1081

AAGCCCTTGG TCCCCCTGGG TCTGAGGGCC CTAGGTCATG GAGAGCCTCA GTCCCCATAA       1141

TGAGGACAGG GTACCATGCC CACCTTTCCT TCAGAACCCT GGGGCCCAGG GCCACCCAGA       1201

GGTAAGAGGA CATTTAGCAT TAGCTCTGTG TGAGCTCCTG CCGGTTTCTT GGCTGTCAGT       1261

CAGTCCCAGA GTGGGGAGGA AGATATGGGT GACCCCCACC CCCCATCTGT GAGCCAAGCC       1321

TCCCTTGTCC CTGGCCTTTG GACCCAGGCA AAGGCTTCTG AGCCCTGGGC AGGGGTGGTG       1381

GGTACCAGAG AATGCTGCCT TCCCCCAAGC CTGCCCCTCT GCCTCATTTT CCTGTAGCTC       1441

CTCTGGTTCT GTTTGCTCAT TGGCCGCTGT GTTCATCCAA GGGGGTTCTC CCAGAAGTGA       1501

GGGGCCTTTC CCTCCATCCC TTGGGGCACG GGGCAGCTGT GCCTGCCCTG CCTCTGCCTG       1561
```

AGGCAGCCGC TCCTGCCTGA GCCTGGACAT GGGGCCCTTC CTTGTGTTGC CAATTTATTA 1621

ACAGCAAATA AACCAATTAA ATGGAGACTA TTAAATAACT TTATTTTAAA AATGAAAAAA 1681

AAAAAAAAAA AAA 1694

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 310 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Leu Lys Ala Asn Ile Pro Glu Val Glu Ala Val Leu Asn Thr Asp
1 5 10 15

Arg Ser Leu Val Cys Asp Gly Lys Arg Gly Leu Leu Thr Arg Leu Leu
20 25 30

Gln Val Met Lys Lys Glu Pro Ala Glu Ser Ser Phe Arg Phe Trp Gln
35 40 45

Ala Arg Ala Val Glu Ser Phe Leu Arg Gly Thr Thr Ser Tyr Ala Asp
50 55 60

Gln Met Phe Leu Leu Lys Arg Gly Leu Leu Glu His Ile Leu Tyr Cys
65 70 75 80

Ile Val Asp Ser Glu Cys Lys Ser Arg Asp Val Leu Gln Ser Tyr Phe
85 90 95

Asp Leu Leu Gly Glu Leu Met Lys Phe Asn Val Asp Ala Phe Lys Arg
100 105 110

Phe Asn Lys Tyr Ile Asn Thr Asp Ala Lys Phe Gln Val Phe Leu Lys
115 120 125

Gln Ile Asn Ser Ser Leu Val Asp Ser Asn Met Leu Val Arg Cys Val
130 135 140

Thr Leu Ser Leu Asp Arg Phe Glu Asn Gln Val Asp Met Lys Val Ala
145 150 155 160

Glu Val Leu Ser Glu Cys Arg Leu Leu Ala Tyr Ile Ser Gln Val Pro
165 170 175

Thr Gln Met Ser Phe Leu Phe Arg Leu Ile Asn Ile Ile His Val Gln
180 185 190

Thr Leu Thr Gln Glu Asn Val Ser Cys Leu Asn Thr Ser Leu Val Ile
195 200 205

Leu Met Leu Ala Arg Arg Lys Glu Arg Leu Pro Leu Tyr Leu Arg Leu
210 215 220

Leu Gln Arg Met Glu His Ser Lys Lys Tyr Pro Gly Phe Leu Leu Asn
225 230 235 240

Asn Phe His Asn Leu Leu Arg Phe Trp Gln Gln His Tyr Leu His Lys
245 250 255

Asp Lys Asp Ser Thr Cys Leu Glu Asn Ser Ser Cys Ile Ser Phe Ser

```
            260                 265                 270
Tyr Trp Lys Glu Thr Val Ser Ile Leu Leu Asn Pro Asp Arg Gln Ser
        275                 280                 285
Pro Ser Ala Leu Val Ser Tyr Ile Glu Glu Pro Tyr Met Asp Ile Asp
    290                 295                 300
Arg Asp Phe Thr Glu Glu
305                 310
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2735 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..1822

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
G GAG ATC AGT CGG AAG GTG TAC AAG GGA ATG TTA GAC CTC CTC AAG          46
  Glu Ile Ser Arg Lys Val Tyr Lys Gly Met Leu Asp Leu Leu Lys
    1               5                  10                  15

TGT ACA GTC CTC AGC TTG GAG CAG TCC TAT GCC CAC GCG GGT CTG GGT        94
Cys Thr Val Leu Ser Leu Glu Gln Ser Tyr Ala His Ala Gly Leu Gly
                20                  25                  30

GGC ATG GCC AGC ATC TTT GGG CTT TTG GAG ATT GCC CAG ACC CAC TAC       142
Gly Met Ala Ser Ile Phe Gly Leu Leu Glu Ile Ala Gln Thr His Tyr
            35                  40                  45

TAT AGT AAA GAA CCA GAC AAG CGG AAG AGA AGT CCA ACA GAA AGT GTA       190
Tyr Ser Lys Glu Pro Asp Lys Arg Lys Arg Ser Pro Thr Glu Ser Val
        50                  55                  60

AAT ACC CCA GTT GGC AAG GAT CCT GGC CTA GCT GGG CGG GGG GAC CCA       238
Asn Thr Pro Val Gly Lys Asp Pro Gly Leu Ala Gly Arg Gly Asp Pro
    65                  70                  75

AAG GCT ATG GCA CAA CTG AGA GTT CCA CAA CTG GGA CCT CGG GCA CCA       286
Lys Ala Met Ala Gln Leu Arg Val Pro Gln Leu Gly Pro Arg Ala Pro
80                  85                  90                  95

AGT GCC ACA GGA AAG GGT CCT AAG GAA CTG GAC ACC AGA AGT TTA AAG       334
Ser Ala Thr Gly Lys Gly Pro Lys Glu Leu Asp Thr Arg Ser Leu Lys
                100                 105                 110

GAA GAA AAT TTT ATA GCA TCT ATT GGG CCT GAA GTA ATC AAA CCT GTC       382
Glu Glu Asn Phe Ile Ala Ser Ile Gly Pro Glu Val Ile Lys Pro Val
            115                 120                 125

TTT GAC CTT GGT GAG ACA GAG GAG AAA AAG TCC CAG ATC AGC GCA GAC       430
Phe Asp Leu Gly Glu Thr Glu Glu Lys Lys Ser Gln Ile Ser Ala Asp
        130                 135                 140
```

```
AGT GGT GTG AGC CTG ACG TCT AGT TCC CAG AGG ACT GAT CAA GAC TCT        478
Ser Gly Val Ser Leu Thr Ser Ser Ser Gln Arg Thr Asp Gln Asp Ser
    145                 150                 155

GTC ATC GGC GTG AGT CCA GCT GTT ATG ATC CGC AGC TCA AGT CAG GAT        526
Val Ile Gly Val Ser Pro Ala Val Met Ile Arg Ser Ser Ser Gln Asp
160                 165                 170                 175

TCT GAA GTT AGC ACC GTG GTG AGT AAT AGC TCT GGA GAG ACC CTT GGA        574
Ser Glu Val Ser Thr Val Val Ser Asn Ser Ser Gly Glu Thr Leu Gly
                180                 185                 190

GCT GAC AGT GAC TTG AGC AGC AAT GCA GGT GAT GGA CCA GGT GGC GAG        622
Ala Asp Ser Asp Leu Ser Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu
            195                 200                 205

GGC AGT GTT CAC CTG GCA AGC TCT CGG GGC ACT TTG TCT GAT AGT GAA        670
Gly Ser Val His Leu Ala Ser Ser Arg Gly Thr Leu Ser Asp Ser Glu
        210                 215                 220

ATT GAG ACC AAC TCT GCC ACA AGC ACC ATC TTT GGT AAA GCC CAC AGC        718
Ile Glu Thr Asn Ser Ala Thr Ser Thr Ile Phe Gly Lys Ala His Ser
    225                 230                 235

TTG AAG CCA AGC ATA AAG GAG AAG CTG GCA GGC AGC CCC ATT CGT ACT        766
Leu Lys Pro Ser Ile Lys Glu Lys Leu Ala Gly Ser Pro Ile Arg Thr
240                 245                 250                 255

TCT GAA GAT GTG AGC CAG CGA GTC TAT CTC TAT GAG GGA CTC CTA GGC        814
Ser Glu Asp Val Ser Gln Arg Val Tyr Leu Tyr Glu Gly Leu Leu Gly
                260                 265                 270

AAA GAG CGT TCT ACT TTA TGG GAC CAA ATG CAA TTC TGG GAA GAT GCC        862
Lys Glu Arg Ser Thr Leu Trp Asp Gln Met Gln Phe Trp Glu Asp Ala
            275                 280                 285

TTC TTA GAT GCT GTG ATG TTG GAG AGA GAA GGG ATG GGT ATG GAC CAG        910
Phe Leu Asp Ala Val Met Leu Glu Arg Glu Gly Met Gly Met Asp Gln
        290                 295                 300

GGT CCC CAG GAA ATG ATC GAC AGG TAC CTG TCC CTT GGA GAA CAT GAC        958
Gly Pro Gln Glu Met Ile Asp Arg Tyr Leu Ser Leu Gly Glu His Asp
    305                 310                 315

CGG AAG CGC CTG GAA GAT GAT GAA GAT CGC TTG CTG GCC ACA CTT CTG       1006
Arg Lys Arg Leu Glu Asp Asp Glu Asp Arg Leu Leu Ala Thr Leu Leu
320                 325                 330                 335

CAC AAC CTC ATC TCC TAC ATG CTG CTG ATG AAG GTA AAT AAG AAT GAC       1054
His Asn Leu Ile Ser Tyr Met Leu Leu Met Lys Val Asn Lys Asn Asp
                340                 345                 350

ATC CGC AAG AAG GTG AGG CGC CTA ATG GGA AAG TCG CAC ATT GGG CTT       1102
Ile Arg Lys Lys Val Arg Arg Leu Met Gly Lys Ser His Ile Gly Leu
            355                 360                 365

GTG TAC AGC CAG CAA ATC AAT GAG GTG CTT GAT CAG CTG GCG AAC CTG       1150
Val Tyr Ser Gln Gln Ile Asn Glu Val Leu Asp Gln Leu Ala Asn Leu
        370                 375                 380

AAT GGA CGC GAT CTC TCT ATC TGG TCC AGT GGC AGC CGG CAC ATG AAG       1198
Asn Gly Arg Asp Leu Ser Ile Trp Ser Ser Gly Ser Arg His Met Lys
    385                 390                 395

AAG CAG ACA TTT GTG GTA CAT GCA GGG ACA GAT ACA AAC GGA GAT ATC       1246
Lys Gln Thr Phe Val Val His Ala Gly Thr Asp Thr Asn Gly Asp Ile
400                 405                 410                 415
```

```
TTT TTC ATG GAG GTG TGC GAT GAC TGT GTG GTG TTG CGT AGT AAC ATC      1294
Phe Phe Met Glu Val Cys Asp Asp Cys Val Val Leu Arg Ser Asn Ile
                420                 425                 430

GGA ACA GTG TAT GAG CGC TGG TGG TAC GAG AAG CTC ATC AAC ATG ACC      1342
Gly Thr Val Tyr Glu Arg Trp Trp Tyr Glu Lys Leu Ile Asn Met Thr
            435                 440                 445

TAC TGT CCC AAG ACG AAG GTG TTG TGC TTG TGG CGT AGA AAT GGC TCT      1390
Tyr Cys Pro Lys Thr Lys Val Leu Cys Leu Trp Arg Arg Asn Gly Ser
        450                 455                 460

GAG ACC CAG CTC AAC AAG TTC TAT ACT AAA AAG TGT CGG GAG CTG TAC      1438
Glu Thr Gln Leu Asn Lys Phe Tyr Thr Lys Lys Cys Arg Glu Leu Tyr
    465                 470                 475

TAC TGT GTG AAG GAC AGC ATG GAG CGC GCT GCC GCC CGA CAG CAA AGC      1486
Tyr Cys Val Lys Asp Ser Met Glu Arg Ala Ala Ala Arg Gln Gln Ser
480                 485                 490                 495

ATC AAA CCC GGA CCT GAA TTG GGT GGC GAG TTC CGT GTG CAG GAC CTG      1534
Ile Lys Pro Gly Pro Glu Leu Gly Gly Glu Phe Pro Val Gln Asp Leu
                500                 505                 510

AAG ACT GGT GAG GGT GGC CTG CTG CAG GTG ACC CTG GAA GGG ATC AAC      1582
Lys Thr Gly Glu Gly Gly Leu Leu Gln Val Thr Leu Glu Gly Ile Asn
            515                 520                 525

CTC AAA TTC ATG CAC AAT CAG GTT TTC ATA GAG CTG AAT CAC ATT AAA      1630
Leu Lys Phe Met His Asn Gln Val Phe Ile Glu Leu Asn His Ile Lys
        530                 535                 540

AAG TGC AAT ACA GTT CGA GGC GTC TTT GTC CTG GAG GAA TTT GTT CCT      1678
Lys Cys Asn Thr Val Arg Gly Val Phe Val Leu Glu Glu Phe Val Pro
    545                 550                 555

GAA ATT AAA GAA GTG GTG AGC CAC AAG TAC AAG ACA CCA ATG GCC CAC      1726
Glu Ile Lys Glu Val Val Ser His Lys Tyr Lys Thr Pro Met Ala His
560                 565                 570                 575

GAA ATC TGC TAC TCC GTA TTA TGT CTC TTC TCG TAC GTG GCT GCA GTT      1774
Glu Ile Cys Tyr Ser Val Leu Cys Leu Phe Ser Tyr Val Ala Ala Val
                580                 585                 590

CAT AGC AGT GAG GAA GAT CTC AGA ACC CCG CCC CGG CCT GTC TCT AGC      1822
His Ser Ser Glu Glu Asp Leu Arg Thr Pro Pro Arg Pro Val Ser Ser
            595                 600                 605

TGATGGAGAG GGGCTACGCA GCTGCCCCAG CCCAGGGCAC GCCCCTGGCC CCTTGCTGTT    1882

CCCAAGTGCA CGATGCTGCT GTGACTGAGG AGTGGATGAT GCTCGTGTGT CCTCTGCAAG    1942

CCCCCTGCTG TGGCTTGGTT GGTTACCGGT TATGTGTCCC TCTGAGTGTG TCTTGAGCGT    2002

GTCCACCTTC TCCCTCTCCA CTCCCAGAAG ACCAAACTGC CTTCCCCTCA GGGCTCAAGA    2062

ATGTGTACAG TCTGTGGGGC CGGTGTGAAC CCACTATTTT GTGTCCTTGA GACATTTGTG    2122

TTGTGGTTCC TTGTCCTTGT CCCTGGCGTT ATAACTGTCC ACTGCAAGAG TCTGGCTCTC    2182

CCTTCTCTGT GACCCGGCAT GACTGGGCGC CTGGAGCAGT TTCACTCTGT GAGGAGTGAG    2242

GGAACCCTGG GGCTCACCCT CTCAGAGGAA GGGCACAGAG AGGAAGGGAA GAATTGGGGG    2302

GCAGCCGGAG TGAGTGGCAG CCTCCCTGCT TCCTTCTGCA TTCCCAAGCC GGCAGCTACT    2362

GCCCAGGGCC CGCAGTGTTG GCTGCTGCCT GCCACAGCCT CTGTGACTGC AGTGGAGCGG    2422
```

CGAATTCCCT GTGGCCTGCC ACGCCTTCGG CATCAGAGGA TGGAGTGGTC GAGGCTAGTG 2482

GAGTCCCAGG GACCGCTGGC TGCTCTGCCT GAGCATCAGG GAGGGGGCAG GAAAGACCAA 2542

GCTGGGTTTG CACATCTGTC TGCAGGCTGT CTCTCCAGGC ACGGGGTGTC AGGAGGGAGA 2602

GACAGCCTGG GTATGGGCAA GAAATGACTG TAAATATTTC AGCCCCACAT TATTTATAGA 2662

AAATGTACAG TTGTGTGAAT GTGAAATAAA TGTCCTCAAC TCCCAAAAAA AAAAAAAAAA 2722

AAAAAAAAAA AAA 2735

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 607 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Ile Ser Arg Lys Val Tyr Lys Gly Met Leu Asp Leu Leu Lys Cys
1 5 10 15

Thr Val Leu Ser Leu Glu Gln Ser Tyr Ala His Ala Gly Leu Gly Gly
20 25 30

Met Ala Ser Ile Phe Gly Leu Leu Glu Ile Ala Gln Thr His Tyr Tyr
35 40 45

Ser Lys Glu Pro Asp Lys Arg Lys Arg Ser Pro Thr Glu Ser Val Asn
50 55 60

Thr Pro Val Gly Lys Asp Pro Gly Leu Ala Gly Arg Gly Asp Pro Lys
65 70 75 80

Ala Met Ala Gln Leu Arg Val Pro Gln Leu Gly Pro Arg Ala Pro Ser
85 90 95

Ala Thr Gly Lys Gly Pro Lys Glu Leu Asp Thr Arg Ser Leu Lys Glu
100 105 110

Glu Asn Phe Ile Ala Ser Ile Gly Pro Glu Val Ile Lys Pro Val Phe
115 120 125

Asp Leu Gly Glu Thr Glu Glu Lys Lys Ser Gln Ile Ser Ala Asp Ser
130 135 140

Gly Val Ser Leu Thr Ser Ser Ser Gln Arg Thr Asp Gln Asp Ser Val
145 150 155 160

Ile Gly Val Ser Pro Ala Val Met Ile Arg Ser Ser Ser Gln Asp Ser
165 170 175

Glu Val Ser Thr Val Val Ser Asn Ser Ser Gly Glu Thr Leu Gly Ala
180 185 190

Asp Ser Asp Leu Ser Ser Asn Ala Gly Asp Gly Pro Gly Gly Glu Gly
195 200 205

Ser Val His Leu Ala Ser Ser Arg Gly Thr Leu Ser Asp Ser Glu Ile
210 215 220

Glu Thr Asn Ser Ala Thr Ser Thr Ile Phe Gly Lys Ala His Ser Leu

225 230 235 240

Lys Pro Ser Ile Lys Glu Lys Leu Ala Gly Ser Pro Ile Arg Thr Ser
245 250 255

Glu Asp Val Ser Gln Arg Val Tyr Leu Tyr Glu Gly Leu Leu Gly Lys
260 265 270

Glu Arg Ser Thr Leu Trp Asp Gln Met Gln Phe Trp Glu Asp Ala Phe
275 280 285

Leu Asp Ala Val Met Leu Glu Arg Glu Gly Met Gly Met Asp Gln Gly
290 295 300

Pro Gln Glu Met Ile Asp Arg Tyr Leu Ser Leu Gly Glu His Asp Arg
305 310 315 320

Lys Arg Leu Glu Asp Asp Glu Asp Arg Leu Leu Ala Thr Leu Leu His
325 330 335

Asn Leu Ile Ser Tyr Met Leu Leu Met Lys Val Asn Lys Asn Asp Ile
340 345 350

Arg Lys Lys Val Arg Arg Leu Met Gly Lys Ser His Ile Gly Leu Val
355 360 365

Tyr Ser Gln Gln Ile Asn Glu Val Leu Asp Gln Leu Ala Asn Leu Asn
370 375 380

Gly Arg Asp Leu Ser Ile Trp Ser Ser Gly Ser Arg His Met Lys Lys
385 390 395 400

Gln Thr Phe Val Val His Ala Gly Thr Asp Thr Asn Gly Asp Ile Phe
405 410 415

Phe Met Glu Val Cys Asp Asp Cys Val Val Leu Arg Ser Asn Ile Gly
420 425 430

Thr Val Tyr Glu Arg Trp Trp Tyr Glu Lys Leu Ile Asn Met Thr Tyr
435 440 445

Cys Pro Lys Thr Lys Val Leu Cys Leu Trp Arg Arg Asn Gly Ser Glu
450 455 460

Thr Gln Leu Asn Lys Phe Tyr Thr Lys Lys Cys Arg Glu Leu Tyr Tyr
465 470 475 480

Cys Val Lys Asp Ser Met Glu Arg Ala Ala Ala Arg Gln Gln Ser Ile
485 490 495

Lys Pro Gly Pro Glu Leu Gly Gly Glu Phe Pro Val Gln Asp Leu Lys
500 505 510

Thr Gly Glu Gly Gly Leu Leu Gln Val Thr Leu Glu Gly Ile Asn Leu
515 520 525

Lys Phe Met His Asn Gln Val Phe Ile Glu Leu Asn His Ile Lys Lys
530 535 540

Cys Asn Thr Val Arg Gly Val Phe Val Leu Glu Glu Phe Val Pro Glu
545 550 555 560

Ile Lys Glu Val Val Ser His Lys Tyr Lys Thr Pro Met Ala His Glu
565 570 575

Ile Cys Tyr Ser Val Leu Cys Leu Phe Ser Tyr Val Ala Ala Val His
580 585 590

```
Ser Ser Glu Glu Asp Leu Arg Thr Pro Pro Arg Pro Val Ser Ser
        595                 600                 605
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3225 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..2846

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
CC CAG ACT CGC CCC GCC CCA GAG ACT GCG CCT GCG CGG GCA CGA GAC          47
   Gln Thr Arg Pro Ala Pro Glu Thr Ala Pro Ala Arg Ala Arg Asp
    1               5                  10                  15

ACC CTC TCC GCG ATG ACT GCC AGC TCA GTG GAG CAG CTG CGG AAG GAG          95
Thr Leu Ser Ala Met Thr Ala Ser Ser Val Glu Gln Leu Arg Lys Glu
                20                  25                  30

GGC AAT GAG CTG TTC AAA TGT GGA GAC TAC GGG GGC GCC CTG GCG GCC         143
Gly Asn Glu Leu Phe Lys Cys Gly Asp Tyr Gly Gly Ala Leu Ala Ala
            35                  40                  45

TAC ACT CAG GCC CTG GGT CTG GAC GCG ACG CCC CAG GAC CAG GCC GTT         191
Tyr Thr Gln Ala Leu Gly Leu Asp Ala Thr Pro Gln Asp Gln Ala Val
        50                  55                  60

CTG CAC CGG AAC CGG GCC GCC TGC CAC CTC AAG CTG GAA GAT TAC GAC         239
Leu His Arg Asn Arg Ala Ala Cys His Leu Lys Leu Glu Asp Tyr Asp
    65                  70                  75

AAA GCA GAA ACA GAG GCA TCC AAA GCC ATT GAA AAG GAT GGT GGG GAT         287
Lys Ala Glu Thr Glu Ala Ser Lys Ala Ile Glu Lys Asp Gly Gly Asp
80                  85                  90                  95

GTC AAA GCA CTC TAC CGG CGG AGC CAA GCC CTA GAG AAG CTG GGC CGC         335
Val Lys Ala Leu Tyr Arg Arg Ser Gln Ala Leu Glu Lys Leu Gly Arg
                100                 105                 110

CTG GAC CAG GCT GTC CTT GAC CTG CAG AGA TGT GTG AGC TTG GAG CCC         383
Leu Asp Gln Ala Val Leu Asp Leu Gln Arg Cys Val Ser Leu Glu Pro
            115                 120                 125

AAG AAC AAA GTT TTC CAG GAG GCC TTG CGG AAC ATC GGG GGC CAG ATT         431
Lys Asn Lys Val Phe Gln Glu Ala Leu Arg Asn Ile Gly Gly Gln Ile
        130                 135                 140

CAG GAG AAG GTG CGA TAC ATG TCC TCG ACG GAT GCC AAA GTG GAA CAG         479
Gln Glu Lys Val Arg Tyr Met Ser Ser Thr Asp Ala Lys Val Glu Gln
    145                 150                 155
```

```
ATG TTT CAG ATA CTG TTG GAC CCA GAA GAG AAG GGC ACT GAG AAA AAG                527
Met Phe Gln Ile Leu Leu Asp Pro Glu Glu Lys Gly Thr Glu Lys Lys
160                 165                 170                 175

CAA AAG GCT TCT CAG AAC CTG GTG GTG CTG GCC AGG GAG GAT GCT GGA               575
Gln Lys Ala Ser Gln Asn Leu Val Val Leu Ala Arg Glu Asp Ala Gly
                180                 185                 190

GCG GAG AAG ATC TTC CGG AGT AAT GGG GTT CAG CTC TTG CAA CGT TTA               623
Ala Glu Lys Ile Phe Arg Ser Asn Gly Val Gln Leu Leu Gln Arg Leu
            195                 200                 205

CTG GAC ATG GGA GAG ACT GAC CTC ATG CTG GCG GCT CTG CGT ACG CTG               671
Leu Asp Met Gly Glu Thr Asp Leu Met Leu Ala Ala Leu Arg Thr Leu
        210                 215                 220

GTT GGC ATT TGC TCT GAG CAT CAG TCA CGG ACA GTG GCA ACC CTG AGC               719
Val Gly Ile Cys Ser Glu His Gln Ser Arg Thr Val Ala Thr Leu Ser
    225                 230                 235

ATA CTG GGA ACT CGG CGA GTA GTC TCC ATC CTG GGC GTG GAA AGC CAG               767
Ile Leu Gly Thr Arg Arg Val Val Ser Ile Leu Gly Val Glu Ser Gln
240                 245                 250                 255

GCT GTG TCC CTG GCT GCC TGC CAC CTG CTG CAG GTT ATG TTT GAT GCC               815
Ala Val Ser Leu Ala Ala Cys His Leu Leu Gln Val Met Phe Asp Ala
                260                 265                 270

CTC AAG GAA GGT GTC AAA AAA GGC TTC CGA GGC AAA GAA GGT GCC ATC               863
Leu Lys Glu Gly Val Lys Lys Gly Phe Arg Gly Lys Glu Gly Ala Ile
            275                 280                 285

ATT GTG GAT CCT GCC CGG GAG CTG AAG GTC CTC ATC AGT AAC CTC TTA               911
Ile Val Asp Pro Ala Arg Glu Leu Lys Val Leu Ile Ser Asn Leu Leu
        290                 295                 300

GAT CTG CTG ACA GAG GTG GGG GTC TCT GGC CAA GGC CGA GAC AAT GCC               959
Asp Leu Leu Thr Glu Val Gly Val Ser Gly Gln Gly Arg Asp Asn Ala
    305                 310                 315

CTG ACC CTC CTG ATT AAA GCG GTG CCC CGG AAG TCT CTC AAG GAC CCC              1007
Leu Thr Leu Leu Ile Lys Ala Val Pro Arg Lys Ser Leu Lys Asp Pro
320                 325                 330                 335

AAC AAC AGC CTC ACC CTC TGG GTC ATC GAC CAA GGT CTG AAA AAG ATT              1055
Asn Asn Ser Leu Thr Leu Trp Val Ile Asp Gln Gly Leu Lys Lys Ile
                340                 345                 350

TTG GAA GTG GGG GGC TCT CTA CAG GAC CCT CCT GGG GAG CTC GCA GTG              1103
Leu Glu Val Gly Gly Ser Leu Gln Asp Pro Pro Gly Glu Leu Ala Val
            355                 360                 365

ACC GCA AAC AGC CGC ATG AGC GCC TCT ATT CTC CTC AGC AAG CTC TTT              1151
Thr Ala Asn Ser Arg Met Ser Ala Ser Ile Leu Leu Ser Lys Leu Phe
        370                 375                 380

GAT GAC CTC AAG TGT GAT GCG GAG AGG GAG AAT TTC CAC AGA CTT TGT              1199
Asp Asp Leu Lys Cys Asp Ala Glu Arg Glu Asn Phe His Arg Leu Cys
    385                 390                 395

GAA AAC TAC ATC AAG AGC TGG TTT GAG GGC CAA GGG CTG GCC GGG AAG              1247
Glu Asn Tyr Ile Lys Ser Trp Phe Glu Gly Gln Gly Leu Ala Gly Lys
400                 405                 410                 415

CTA CGG GCC ATC CAG ACG GTG TCC TGC CTC CTG CAG GGC CCA TGT GAC              1295
Leu Arg Ala Ile Gln Thr Val Ser Cys Leu Leu Gln Gly Pro Cys Asp
                420                 425                 430
```

```
GCT GGC AAC CGG GCC TTG GAG CTG AGC GGT GTC ATG GAG AGT GTG ATT      1343
Ala Gly Asn Arg Ala Leu Glu Leu Ser Gly Val Met Glu Ser Val Ile
            435                 440                 445

GCT CTG TGT GCC TCT GAG CAG GAG GAG GAG CAG CTG GTG GCC GTG GAG      1391
Ala Leu Cys Ala Ser Glu Gln Glu Glu Glu Gln Leu Val Ala Val Glu
        450                 455                 460

GCT CTG ATC CAT GCA GCC GGC AAG GCT AAG CGG GCC TCA TTC ATC ACT      1439
Ala Leu Ile His Ala Ala Gly Lys Ala Lys Arg Ala Ser Phe Ile Thr
    465                 470                 475

GCC AAT GGT GTC TCG CTG CTG AAG GAC CTA TAT AAG TGC AGC GAG AAG      1487
Ala Asn Gly Val Ser Leu Leu Lys Asp Leu Tyr Lys Cys Ser Glu Lys
480                 485                 490                 495

GAC AGC ATC CGC ATC CGG GCG CTA GTG GGA CTC TGT AAG CTC GGT TCG      1535
Asp Ser Ile Arg Ile Arg Ala Leu Val Gly Leu Cys Lys Leu Gly Ser
                500                 505                 510

GCT GGA GGG ACT GAC TTC AGC ATG AAG CAG TTT GCT GAA GGC TCC ACT      1583
Ala Gly Gly Thr Asp Phe Ser Met Lys Gln Phe Ala Glu Gly Ser Thr
            515                 520                 525

CTC AAA CTG GCT AAG CAG TGT CGA AAG TGG CTG TGC AAT GAC CAG ATC      1631
Leu Lys Leu Ala Lys Gln Cys Arg Lys Trp Leu Cys Asn Asp Gln Ile
        530                 535                 540

GAC GCA GGC ACT CGG CGC TGG GCA GTG GAG GGC CTG GCT TAC CTG ACC      1679
Asp Ala Gly Thr Arg Arg Trp Ala Val Glu Gly Leu Ala Tyr Leu Thr
    545                 550                 555

TTT GAT GCC GAC GTG AAG GAA GAG TTT GTG GAG GAT GCG GCT GCT CTG      1727
Phe Asp Ala Asp Val Lys Glu Glu Phe Val Glu Asp Ala Ala Ala Leu
560                 565                 570                 575

AAA GCT CTG TTC CAG CTC AGC AGG TTG GAG GAG AGG TCA GTG CTC TTT      1775
Lys Ala Leu Phe Gln Leu Ser Arg Leu Glu Glu Arg Ser Val Leu Phe
                580                 585                 590

GCG GTG GCC TCA GCG CTG GTG AAC TGC ACC AAC AGC TAT GAC TAC GAG      1823
Ala Val Ala Ser Ala Leu Val Asn Cys Thr Asn Ser Tyr Asp Tyr Glu
            595                 600                 605

GAG CCC GAC CCC AAG ATG GTG GAG CTG GCC AAG TAT GCC AAG CAG CAT      1871
Glu Pro Asp Pro Lys Met Val Glu Leu Ala Lys Tyr Ala Lys Gln His
        610                 615                 620

GTG CCC GAG CAG CAC CCC AAG GAC AAG CCA AGC TTC GTG CGG GCT CGG      1919
Val Pro Glu Gln His Pro Lys Asp Lys Pro Ser Phe Val Arg Ala Arg
    625                 630                 635

GTG AAG AAG CTG CTG GCA GCG GGT GTG GTG TCG GCC ATG GTG TGC ATG      1967
Val Lys Lys Leu Leu Ala Ala Gly Val Val Ser Ala Met Val Cys Met
640                 645                 650                 655

GTG AAG ACG GAG AGC CCT GTG CTG ACC AGT TCC TGC AGA GAG CTG CTC      2015
Val Lys Thr Glu Ser Pro Val Leu Thr Ser Ser Cys Arg Glu Leu Leu
                660                 665                 670

TCC AGG GTC TTC TTG GCT TTA GTG GAA GAG GTA GAG GAC CGA GGC ACT      2063
Ser Arg Val Phe Leu Ala Leu Val Glu Glu Val Glu Asp Arg Gly Thr
            675                 680                 685

GTG GTT GCC CAG GGA GGC GGC AGG GCG CTG ATC CCG CTG GCC CTG GAA      2111
Val Val Ala Gln Gly Gly Gly Arg Ala Leu Ile Pro Leu Ala Leu Glu
        690                 695                 700
```

```
GGC ACG GAC GTG GGG CAG ACA AAG GCA GCC CAG GCC CTT GCC AAG CTC      2159
Gly Thr Asp Val Gly Gln Thr Lys Ala Ala Gln Ala Leu Ala Lys Leu
    705                 710                 715

ACC ATC ACC TCC AAC CCG GAG ATG ACC TTC CCT GGC GAG CGG ATC TAT      2207
Thr Ile Thr Ser Asn Pro Glu Met Thr Phe Pro Gly Glu Arg Ile Tyr
720                 725                 730                 735

GAG GTG GTC CGG CCC CTC GTC TCC CTG TTG CAC CTC AAC TGC TCA GGC      2255
Glu Val Val Arg Pro Leu Val Ser Leu Leu His Leu Asn Cys Ser Gly
                740                 745                 750

CTG CAG AAC TTC GAG GCG CTC ATG GCC CTA ACA AAC CTG GCT GGG ATC      2303
Leu Gln Asn Phe Glu Ala Leu Met Ala Leu Thr Asn Leu Ala Gly Ile
            755                 760                 765

AGC GAG AGG CTC CGG CAG AAG ATC CTG AAG GAG AAG GCT GTG CCC ATG      2351
Ser Glu Arg Leu Arg Gln Lys Ile Leu Lys Glu Lys Ala Val Pro Met
        770                 775                 780

ATA GAA GGC TAC ATG TTT GAG GAG CAT GAG ATG ATC CGC CGG GCA GCC      2399
Ile Glu Gly Tyr Met Phe Glu Glu His Glu Met Ile Arg Arg Ala Ala
    785                 790                 795

ACG GAG TGC ATG TGT AAC TTG GCC ATG AGC AAG GAG GTG CAG GAC CTC      2447
Thr Glu Cys Met Cys Asn Leu Ala Met Ser Lys Glu Val Gln Asp Leu
800                 805                 810                 815

TTC GAA GCC CAG GGC AAT GAC CGA CTG AAG CTG CTG GTG CTG TAC AGT      2495
Phe Glu Ala Gln Gly Asn Asp Arg Leu Lys Leu Leu Val Leu Tyr Ser
                820                 825                 830

GGA GAG GAT GAT GAG CTG CTA CAG CGG GCA GCT GCC GGG GGC TTG GCC      2543
Gly Glu Asp Asp Glu Leu Leu Gln Arg Ala Ala Ala Gly Gly Leu Ala
            835                 840                 845

ATG CTT ACC TCC ATG CGG CCC ACG CTC TGC AGC CGC ATT CCC CAA GTG      2591
Met Leu Thr Ser Met Arg Pro Thr Leu Cys Ser Arg Ile Pro Gln Val
        850                 855                 860

ACC ACA CAC TGG CTG GAG ATC CTG CAG GCC CTG CTT CTG AGC TCC AAC      2639
Thr Thr His Trp Leu Glu Ile Leu Gln Ala Leu Leu Leu Ser Ser Asn
    865                 870                 875

CAG GAG CTG CAG CAC CGG GGT GCT GTG GTG GTG CTG AAC ATG GTG GAG      2687
Gln Glu Leu Gln His Arg Gly Ala Val Val Val Leu Asn Met Val Glu
880                 885                 890                 895

GCC TCG AGG GAG ATT GCC AGC ACC CTG ATG GAG AGT GAG ATG ATG GAG      2735
Ala Ser Arg Glu Ile Ala Ser Thr Leu Met Glu Ser Glu Met Met Glu
                900                 905                 910

ATC TTG TCA GTG CTA GCT AAG GGT GAC CAC AGC CCT GTC ACA AGG GCT      2783
Ile Leu Ser Val Leu Ala Lys Gly Asp His Ser Pro Val Thr Arg Ala
            915                 920                 925

GCT GCA GCC TGC CTG GAC AAA GCA GTG GAA TAT GGG CTT ATC CAA CCC      2831
Ala Ala Ala Cys Leu Asp Lys Ala Val Glu Tyr Gly Leu Ile Gln Pro
        930                 935                 940

AAC CAA GAT GGA GAG TGAGGGGGTT GTCCCTGGGC CCAAGGCTCA TGCACACGCT      2886
Asn Gln Asp Gly Glu
    945

ACCTATTGTG GCACGGAGAG TAAGGACGGA AGCAGCTTTG GCTGGTGGTG GCTGGCATGC    2946

CCAATACTCT TGCCCATCCT CGCTTGCTGC CCTAGGATGT CCTCTGTTCT GAGTCAGCGG    3006
```

CCACGTTCAG TCACACAGCC CTGCTTGGCC AGCACTGCCT GCAGCCTCAC TCAGAGGGGC 3066

CCTTTTTCTG TACTACTGTA GTCAGCTGGG AATGGGGAAG GTGCATCCCA ACACAGCCTG 3126

TGGATCCTGG GGCATTTGGA AGGGCGCACA CATCAGCAGC CTCACCAGCT GTGAGCCTGC 3186

TATCAGGCCT GCCCCTCCAA TAAAAGTGTG TAGAACTCC 3225

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 948 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Thr Arg Pro Ala Pro Glu Thr Ala Pro Ala Arg Ala Arg Asp Thr
1 5 10 15

Leu Ser Ala Met Thr Ala Ser Ser Val Glu Gln Leu Arg Lys Glu Gly
20 25 30

Asn Glu Leu Phe Lys Cys Gly Asp Tyr Gly Gly Ala Leu Ala Ala Tyr
35 40 45

Thr Gln Ala Leu Gly Leu Asp Ala Thr Pro Gln Asp Gln Ala Val Leu
50 55 60

His Arg Asn Arg Ala Ala Cys His Leu Lys Leu Glu Asp Tyr Asp Lys
65 70 75 80

Ala Glu Thr Glu Ala Ser Lys Ala Ile Glu Lys Asp Gly Gly Asp Val
85 90 95

Lys Ala Leu Tyr Arg Arg Ser Gln Ala Leu Glu Lys Leu Gly Arg Leu
100 105 110

Asp Gln Ala Val Leu Asp Leu Gln Arg Cys Val Ser Leu Glu Pro Lys
115 120 125

Asn Lys Val Phe Gln Glu Ala Leu Arg Asn Ile Gly Gly Gln Ile Gln
130 135 140

Glu Lys Val Arg Tyr Met Ser Ser Thr Asp Ala Lys Val Glu Gln Met
145 150 155 160

Phe Gln Ile Leu Leu Asp Pro Glu Glu Lys Gly Thr Glu Lys Lys Gln
165 170 175

Lys Ala Ser Gln Asn Leu Val Val Leu Ala Arg Glu Asp Ala Gly Ala
180 185 190

Glu Lys Ile Phe Arg Ser Asn Gly Val Gln Leu Leu Gln Arg Leu Leu
195 200 205

Asp Met Gly Glu Thr Asp Leu Met Leu Ala Ala Leu Arg Thr Leu Val
210 215 220

Gly Ile Cys Ser Glu His Gln Ser Arg Thr Val Ala Thr Leu Ser Ile
225 230 235 240

Leu Gly Thr Arg Arg Val Val Ser Ile Leu Gly Val Glu Ser Gln Ala
245 250 255

Val Ser Leu Ala Ala Cys His Leu Leu Gln Val Met Phe Asp Ala Leu
260 265 270

Lys Glu Gly Val Lys Lys Gly Phe Arg Gly Lys Glu Gly Ala Ile Ile
275 280 285

Val Asp Pro Ala Arg Glu Leu Lys Val Leu Ile Ser Asn Leu Leu Asp
290 295 300

Leu Leu Thr Glu Val Gly Val Ser Gly Gln Gly Arg Asp Asn Ala Leu
305 310 315 320

Thr Leu Leu Ile Lys Ala Val Pro Arg Lys Ser Leu Lys Asp Pro Asn
325 330 335

Asn Ser Leu Thr Leu Trp Val Ile Asp Gln Gly Leu Lys Lys Ile Leu
340 345 350

Glu Val Gly Gly Ser Leu Gln Asp Pro Pro Gly Glu Leu Ala Val Thr
355 360 365

Ala Asn Ser Arg Met Ser Ala Ser Ile Leu Leu Ser Lys Leu Phe Asp
370 375 380

Asp Leu Lys Cys Asp Ala Glu Arg Glu Asn Phe His Arg Leu Cys Glu
385 390 395 400

Asn Tyr Ile Lys Ser Trp Phe Glu Gly Gln Gly Leu Ala Gly Lys Leu
405 410 415

Arg Ala Ile Gln Thr Val Ser Cys Leu Leu Gln Gly Pro Cys Asp Ala
420 425 430

Gly Asn Arg Ala Leu Glu Leu Ser Gly Val Met Glu Ser Val Ile Ala
435 440 445

Leu Cys Ala Ser Glu Gln Glu Glu Glu Gln Leu Val Ala Val Glu Ala
450 455 460

Leu Ile His Ala Ala Gly Lys Ala Lys Arg Ala Ser Phe Ile Thr Ala
465 470 475 480

Asn Gly Val Ser Leu Leu Lys Asp Leu Tyr Lys Cys Ser Glu Lys Asp
485 490 495

Ser Ile Arg Ile Arg Ala Leu Val Gly Leu Cys Lys Leu Gly Ser Ala
500 505 510

Gly Gly Thr Asp Phe Ser Met Lys Gln Phe Ala Glu Gly Ser Thr Leu
515 520 525

Lys Leu Ala Lys Gln Cys Arg Lys Trp Leu Cys Asn Asp Gln Ile Asp
530 535 540

Ala Gly Thr Arg Arg Trp Ala Val Glu Gly Leu Ala Tyr Leu Thr Phe
545 550 555 560

Asp Ala Asp Val Lys Glu Glu Phe Val Glu Asp Ala Ala Ala Leu Lys
565 570 575

Ala Leu Phe Gln Leu Ser Arg Leu Glu Glu Arg Ser Val Leu Phe Ala
580 585 590

Val Ala Ser Ala Leu Val Asn Cys Thr Asn Ser Tyr Asp Tyr Glu Glu
595 600 605

Pro Asp Pro Lys Met Val Glu Leu Ala Lys Tyr Ala Lys Gln His Val

```
    610                 615                 620

Pro Glu Gln His Pro Lys Asp Lys Pro Ser Phe Val Arg Ala Arg Val
625                 630                 635                 640

Lys Lys Leu Leu Ala Ala Gly Val Val Ser Ala Met Val Cys Met Val
                645                 650                 655

Lys Thr Glu Ser Pro Val Leu Thr Ser Ser Cys Arg Glu Leu Leu Ser
            660                 665                 670

Arg Val Phe Leu Ala Leu Val Glu Glu Val Glu Asp Arg Gly Thr Val
        675                 680                 685

Val Ala Gln Gly Gly Gly Arg Ala Leu Ile Pro Leu Ala Leu Glu Gly
    690                 695                 700

Thr Asp Val Gly Gln Thr Lys Ala Ala Gln Ala Leu Ala Lys Leu Thr
705                 710                 715                 720

Ile Thr Ser Asn Pro Glu Met Thr Phe Pro Gly Glu Arg Ile Tyr Glu
                725                 730                 735

Val Val Arg Pro Leu Val Ser Leu Leu His Leu Asn Cys Ser Gly Leu
            740                 745                 750

Gln Asn Phe Glu Ala Leu Met Ala Leu Thr Asn Leu Ala Gly Ile Ser
        755                 760                 765

Glu Arg Leu Arg Gln Lys Ile Leu Lys Glu Lys Ala Val Pro Met Ile
    770                 775                 780

Glu Gly Tyr Met Phe Glu Glu His Glu Met Ile Arg Arg Ala Ala Thr
785                 790                 795                 800

Glu Cys Met Cys Asn Leu Ala Met Ser Lys Glu Val Gln Asp Leu Phe
                805                 810                 815

Glu Ala Gln Gly Asn Asp Arg Leu Lys Leu Leu Val Leu Tyr Ser Gly
            820                 825                 830

Glu Asp Asp Glu Leu Leu Gln Arg Ala Ala Ala Gly Gly Leu Ala Met
        835                 840                 845

Leu Thr Ser Met Arg Pro Thr Leu Cys Ser Arg Ile Pro Gln Val Thr
    850                 855                 860

Thr His Trp Leu Glu Ile Leu Gln Ala Leu Leu Leu Ser Ser Asn Gln
865                 870                 875                 880

Glu Leu Gln His Arg Gly Ala Val Val Val Leu Asn Met Val Glu Ala
                885                 890                 895

Ser Arg Glu Ile Ala Ser Thr Leu Met Glu Ser Glu Met Met Glu Ile
            900                 905                 910

Leu Ser Val Leu Ala Lys Gly Asp His Ser Pro Val Thr Arg Ala Ala
        915                 920                 925

Ala Ala Cys Leu Asp Lys Ala Val Glu Tyr Gly Leu Ile Gln Pro Asn
    930                 935                 940

Gln Asp Gly Glu
945
```

CLAIMS

What is claimed is:

1. A composition comprising an isolated polynucleotide encoding a protein having TNF-R1-DD ligand protein activity.

2. The composition of claim 1 wherein said polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

3. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

4. A composition of claim 1 wherein said polynucleotide is operably linked to an expression control sequence.

5. A host cell transformed with a composition of claim 4.

6. The host cell of claim 5, wherein said cell is a mammalian cell.

7. A process for producing an TNF-R1-DD ligand protein, which comprises:

(a) growing a culture of the host cell of claim 5 in a suitable culture medium; and

(b) purifying the TNF-R1-DD ligand protein from the culture.

8. A composition comprising a protein having TNF-R1-DD ligand protein activity.

9. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2; and

(b) fragments of the amino acid sequence of SEQ ID NO:2;

said protein being substantially free from other mammalian proteins.

10. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:4; and

(b) fragments of the amino acid sequence of SEQ ID NO:4;

said protein being substantially free from other mammalian proteins.

11. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:6; and

(b) fragments of the amino acid sequence of SEQ ID NO:6;

said protein being substantially free from other mammalian proteins.

12. The composition of claim 8, further comprising a pharmaceutically acceptable carrier.

13. A composition comprising an antibody which specifically reacts with the TNF-R1-DD ligand protein of claim 8.

14. A method of identifying an inhibitor of TNF-R death domain binding which comprises:

(a) combining an TNF-R death domain protein with a composition of claim 8, said combination forming a first binding mixture;

(b) measuring the amount of binding between the TNF-R death domain protein and the TNF-R1-DD ligand protein in the first binding mixture;

(c) combining a compound with the TNF-R death domain protein and an TNF-R1-DD ligand protein to form a second binding mixture;

(d) measuring the amount of binding in the second binding mixture; and

(e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture:

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the amount of binding of the second binding mixture occurs.

15. The method of claim 14 wherein said TNF-R1-DD ligand protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) fragments of the amino acid sequence of SEQ ID NO:2;

(c) the amino acid sequence of SEQ ID NO:4;

(d) fragments of the amino acid sequence of SEQ ID NO:4:

(e) the amino acid sequence of SEQ ID NO:6;

(f) fragments of the amino acid sequence of SEQ ID NO:6:

(g) the amino acid sequence of SEQ ID NO:8; and

(h) fragments of the amino acid sequence of SEQ ID NO:8.

16. A method of preventing or ameliorating an inflammatory condition which comprises administering a therapeutically effective amount of a composition of claim 12.

17. TNF-R1-DD ligand protein produced according to the method of claim 7.

18. A method of inhibiting TNF-R death domain binding comprising administering a therapeutically effective amount of a composition of claim 12.

19. A method of preventing or ameliorating an inflammatory condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a protein selected from the group consisting of IGFBP-5 and fragments thereof having TNF-R1-DD ligand protein activity.

20. A method of inhibiting TNF-R death domain binding comprising administering to a mammalian subject a therapeutically effective amount of a composition comprising a pharmaceutically acceptable carrier and a protein selected from the group consisting of IGFBP-5 and fragments thereof having TNF-R1-DD ligand protein activity.

21. A composition comprising an inhibitor identified according to the method of claim 14.

22. The composition of claim 21 further comprising a pharmaceutically acceptable carrier.

23. A method of preventing or ameliorating an inflammatory condition comprising administering to a mammalian subject a therapeutically effective amount of the composition of claim 22.

24. A method of inhibiting TNF-R death domain binding comprising administering to a mammalian subject a therapeutically effective amount of the composition of claim 22.

25. A composition comprising a pharmaceutically acceptable carrier and a protein selected from the group consisting of IGFBP-5 and fragments thereof having TNF-R1-DD ligand protein activity.

26. A method of identifying an inhibitor of TNF-R death domain binding which comprises:

(a) transforming a cell with a first polynucleotide encoding an TNF-R death domain protein, a second polynucleotide encoding an TNF-R1-DD ligand protein, and at least one reporter gene, wherein the expression of the reporter gene is regulated by the binding of the TNF-R1-DD ligand protein encoded by the second polynucleotide to the TNF-R death domain protein encoded by the first polynucleotide;

(b) growing the cell in the presence of and in the absence of a compound; and

(c) comparing the degree of expression of the reporter gene in the presence of and in the absence of the compound;

wherein the compound is capable of inhibiting TNF-R death domain binding when a decrease in the degree of expression of the reporter gene occurs.

27. The method of claim 26 wherein the second polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 2 to nucleotide 1231;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1, which encodes a protein having TNF-R1-DD ligand protein activity;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:2;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 and having TNF-R1-DD ligand protein activity;

(e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 2 to nucleotide 415;

(f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3, which encodes a protein having TNF-R1-DD ligand protein activity;

(g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:4;

(h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 and having TNF-R1-DD ligand protein activity;

(i) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 2 to nucleotide 559;

(j) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5, which encodes a protein having TNF-R1-DD ligand protein activity;

(k) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:6;

(l) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 and having TNF-R1-DD ligand protein activity;

(m) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 57 to nucleotide 875;

(n) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7, which encodes a protein having TNF-R1-DD ligand protein activity;

(o) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:8;

(p) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 and having TNF-R1-DD ligand protein activity; and

(q) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(p). which encodes a protein having TNF-R1-DD ligand protein activity.

28. The method of claim 26 wherein the cell is a yeast cell.

29. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

30. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12; and

(e) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(d).

31. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10: and

(b) fragments of the amino acid sequence of SEQ ID NO:10;

said protein being substantially free from other mammalian proteins.

32. The composition of claim 8 wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:12: and

(b) fragments of the amino acid sequence of SEQ ID NO:12;

said protein being substantially free from other mammalian proteins.

33. The method of claim 14 wherein said TNF-R1-DD ligand protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:10;

(b) fragments of the amino acid sequence of SEQ ID NO:10;

(c) the amino acid sequence of SEQ ID NO:12; and

(d) fragments of the amino acid sequence of SEQ ID NO:12.

34. The method of claim 26 wherein the second polynucleotide is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 2 to nucleotide 931;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9, which encodes a protein having TNF-R1-DD ligand protein activity;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:10;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 and having TNF-R1-DD ligand protein activity;

(e) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 2 to nucleotide 1822;

(f) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11, which encodes a protein having TNF-R1-DD ligand protein activity;

(g) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:12; and

(h) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 and having TNF-R1-DD ligand protein activity; and

(i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), which encodes a protein having TNF-R1-DD ligand protein activity.

35. The composition of claim 1 wherein said polynucleotide sequence is selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 3 to nucleotide 2846;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:13;

(c) a polynucleotide encoding an TNF-R1-DD ligand protein comprising the amino acid sequence of SEQ ID NO:14;

(d) a polynucleotide encoding an TNF-R1-DD ligand protein comprising a fragment of the amino acid sequence of SEQ ID NO:14; and

Fig. 1

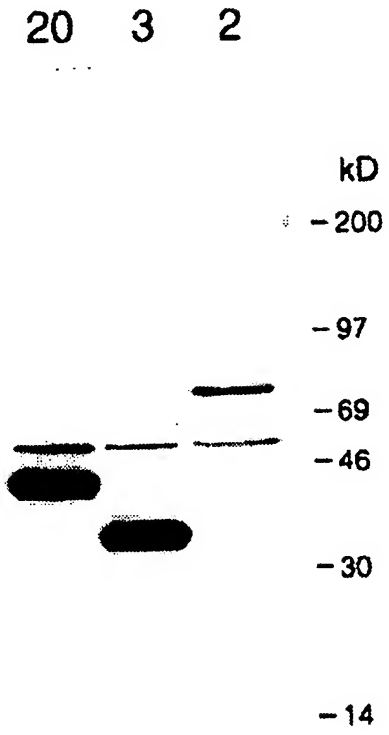


Fig. 2

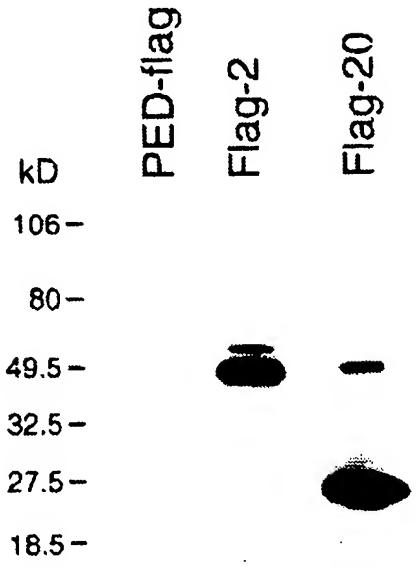


Fig. 3A

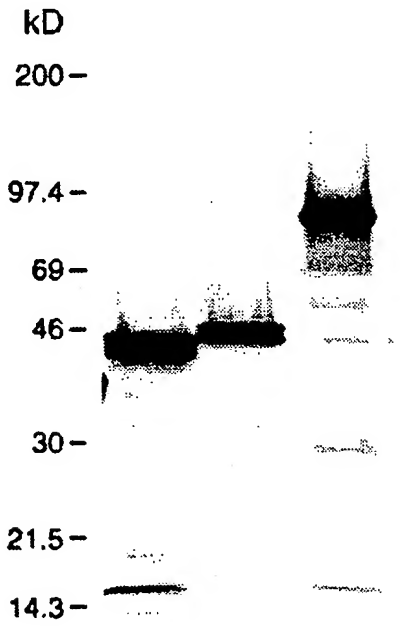


Fig. 3B

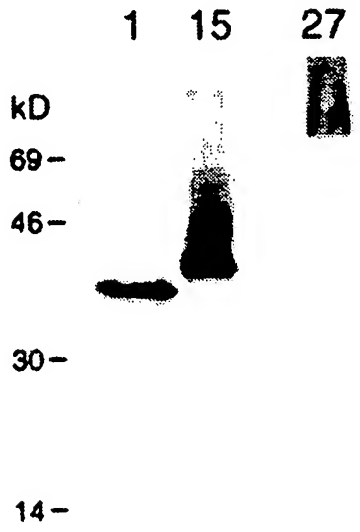
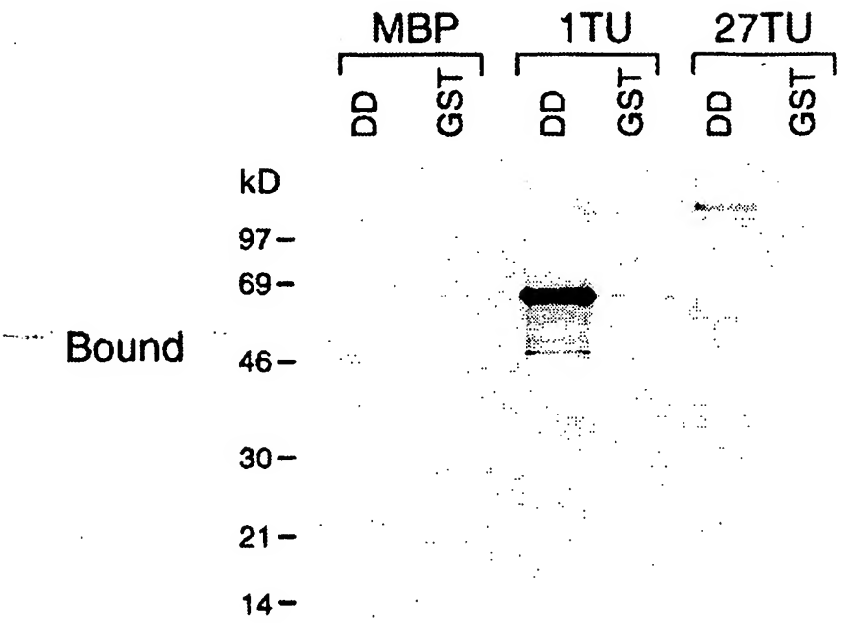


Fig. 4


MBP
1TU
27TU
DD
GST
DD
GST
DD
GST
kD
97
69
46
30
21
14
Bound

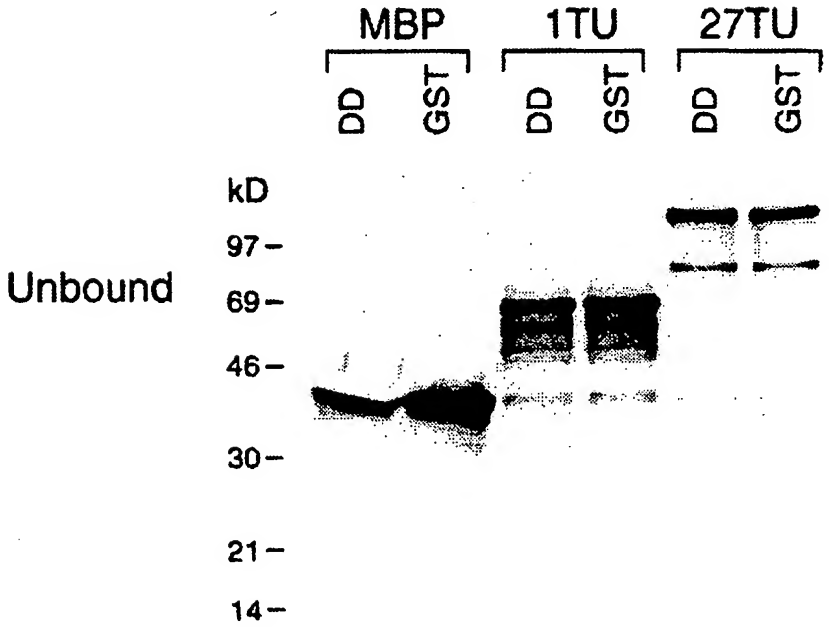

MBP
1TU
27TU
DD
GST
DD
GST
DD
GST
kD
97
69
46
30
21
14
Unbound

Fig. 5

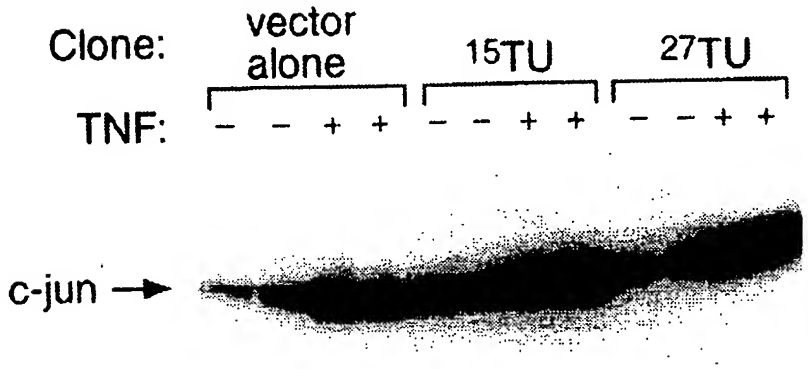


Fig. 6

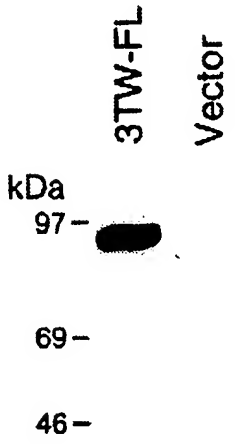
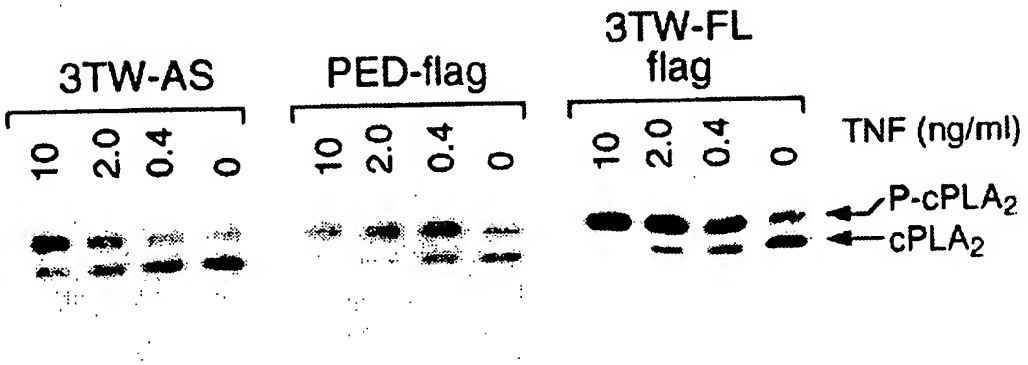


Fig. 7



INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 95/12724

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/47 C07K16/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages.	Relevant to claim No.
X	WO,A,94 01548 (MEDICAL RESEARCH COUNCIL) 20 January 1994 see sequence 494 and passim ---	1,3-8, 26-28,35
X	CELL, vol. 78, 26 August 1994 NA US, pages 681-692, M ROTHE ET AL. 'A novel family of putative signal transducers associated with the cytoplasmic domain of the 75 kDa tumor necrosis factor receptor' see the whole document --- -/--	1,4-8

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 1996	15. 03. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 95/12724

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 36, 9 August 1994 MD US, pages 22492-22495, H Y SONG ET AL. 'Aggregation of the intracellular domain of the type 1 tumor necrosis factor receptor defined by the two-hybrid system' see the whole document ---	1,4-8
A	CELL, vol. 74, no. 8, 10 September 1993 NA US, pages 845-853, L A TARTAGLIA ET AL. 'A novel domain within the 55 kD TNF receptor signals cell death' cited in the application see the whole document ---	1
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 1, 6 January 1995 MD US, pages 387-391, M P BOLDIN ET AL. 'Self-association of the "Death-Domains" of the p55 Tumor Necrosis factor (TNF) receptor and Fas/Apo1 prompts signalling for TNF and Fas/Apo1 effects' see the whole document ---	1
P,X	CELL, vol. 81, 19 May 1995 NA US, pages 495-504, H HSU ET AL. 'The TNF receptor 1-associated protein TRADD signals cell death and NF-kappaB association' see the whole document ---	1
P,X	FEBS LETTERS, vol. 367, 1995 AMSTERDAM NL, pages 39-44, M P BOLDIN ET AL. 'A protein related to a proteasome subunit binds to the intracellular domain of the p55 TNF receptor upstream to its "death domain"' see the whole document ---	1
P,X	PROTEIN ENGINEERING, vol. 8 supplement, 1995 ENGLAND GB, page 90 MMY WAYE ET AL. 'Gene expression of adult human heart as revealed by random sequencing of cDNA library' see the whole document --- -/--	1

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	COMPTES RENDUS DES SEANCES DE L'ACADEMIE DES SCIENCES SERIE III: SCIENCES DE LA VIE., vol. 318, 1995 MONTREUIL FR, pages 263-272, C AUFFRAY ET AL. 'IMAGE: intégration au niveau moléculaire de l'analyse du génome humain et de son expression' see the whole document	1
X	WO,A,94 10207 (CHIRON) 11 May 1994 see the whole document	1,11, 13-17, 19-28
X	WO,A,92 14834 (WHITTIER INSTITUTE) 3 September 1992 see the whole document	1,11, 13-17, 19-28
X	WO,A,92 03471 (CHIRON) 5 March 1992 see the whole document	1,11, 13-17, 19-28
X	WO,A,92 03470 (CHIRON) 5 March 1992 see the whole document	1,11, 13-17, 19-28

2

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/US95/12724

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 16,18-20,23-24
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims refer to a method of treatment of the human body, the search was carried out and based on the alleged effects of the products.
See also continuation sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US95/12724

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

No other distinguishing feature has been provided for the problems of claim 1 than their ability to bind the death domain of the TNF-R1 receptor.
This makes a complete search impossible for economical reasons.

The search was limited to real examples (seq. 1-16) provided by the applicant.

Claims searched incompletely: 1,4-8,12-14,16-18,21-24,26,28

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 95/12724

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9401548	20-01-94	AU-B-	4512193	31-01-94
		EP-A-	0587279	16-03-94
WO-A-9410207	11-05-94	AU-B-	5665294	24-05-94
		CA-A-	2148685	11-05-94
		EP-A-	0668879	30-08-95
WO-A-9214834	03-09-92	NONE		
WO-A-9203471	05-03-92	CA-A-	2090702	01-03-92
		CA-A-	2090703	01-03-92
		CA-A-	2090704	01-03-92
		CA-A-	2090705	01-03-92
		EP-A-	0546053	16-06-93
		EP-A-	0546074	16-06-93
		EP-A-	0546081	16-06-93
		EP-A-	0546110	16-06-93
		JP-T-	6503709	28-04-94
		JP-T-	6503947	12-05-94
		JP-T-	6503711	28-04-94
		JP-T-	6503949	12-05-94
		WO-A-	9203469	05-03-92
		WO-A-	9203152	05-03-92
		WO-A-	9203470	05-03-92
		US-A-	5212074	18-05-93
WO-A-9203470	05-03-92	CA-A-	2090702	01-03-92
		CA-A-	2090703	01-03-92
		CA-A-	2090704	01-03-92
		CA-A-	2090705	01-03-92
		EP-A-	0546053	16-06-93
		EP-A-	0546074	16-06-93
		EP-A-	0546081	16-06-93
		EP-A-	0546110	16-06-93
		JP-T-	6503709	28-04-94
		JP-T-	6503947	12-05-94
		JP-T-	6503711	28-04-94
		JP-T-	6503949	12-05-94
		WO-A-	9203469	05-03-92
		WO-A-	9203152	05-03-92

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern nal Application No
PCT/US 95/12724

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9203470		WO-A- 9203471	05-03-92
		US-A- 5212074	18-05-93